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Alpha-v-containing integrins are host receptors for the *Plasmodium falciparum* sporozoite surface protein, TRAP

Kirsten Dundas, Melanie J. Shears, Yi Sun, Christine S. Hopp, Cecile Crosnier, Tom Metcalf, Gareth Girling, Photini Sinnis, Oliver Billker, and Gavin J. Wright

Malaria-causing *Plasmodium* sporozoites are deposited in the dermis by the bite of an infected mosquito and move by gliding motility to the liver where they invade and develop within host hepatocytes. Although extracellular interactions between *Plasmodium* sporozoite ligands and host receptors provide important guidance cues for productive infection and are good vaccine targets, these interactions remain largely uncharacterized. Thrombospondin-related anonymous protein (TRAP) is a parasite cell surface ligand that is essential for both gliding motility and invasion because it couples the extracellular binding of host receptors to the parasite cytoplasmic actinomyosin motor; however, the molecular nature of the host TRAP receptors is poorly defined. Here, we use a systematic extracellular protein interaction screening approach to identify the integrin αvβ3 as a directly interacting host receptor for *Plasmodium falciparum* TRAP. Biochemical characterization of the interaction suggests a two-site binding model, requiring contributions from both the von Willebrand factor A domain and the RGD motif of TRAP for integrin binding. We show that TRAP binding to cells is promoted in the presence of intergenerativating proadhesive Mn^{2+} ions, and that cells genetically targeted so that they lack cell surface expression of the integrin αv subunit are no longer able to bind TRAP. *P. falciparum* sporozoites moved with greater speed in the dermis of Itgb3-deficient mice, suggesting that the interaction has a role in sporozoite migration. The identification of the integrin αvβ3 as the host receptor for TRAP provides an important demonstration of a sporozoite surface ligand that directly interacts with host receptors.

Malaria is an infectious tropical disease caused by parasites belonging to the genus *Plasmodium* and is responsible for almost half a million deaths annually (1). Infections are initiated when an anopheles mosquito takes a blood meal and deposits the sporozoite form of the parasite within the dermis. Sporozoites are independently motile and disperse from the site of inoculation, enter the circulation, and invade and develop within the liver to continue their life cycle (2). The sporozoite stage is considered an attractive target for vaccines because this stage of the infection is asymptomatic and extracellular sporozoites, which are few in number, are directly exposed to host antibodies.

*Plasmodium* parasites move by gliding motility, a form of movement which requires anchorage on an extracellular substrate and is characterized by a lack of any locomotory organelles and no overt change in cell shape (3). The molecular machinery that is responsible for this gliding behavior involves a protein complex that couples a force-generating cytoplasmic actinomyosin motor to a membrane-spanning “invasive” belonging to the thrombospondin-related anonymous protein (TRAP) family whose interactions with extracellular ligands provide the necessary traction to power movement and invasion (4). *Plasmodium* genomes encode several different members of the TRAP family that are largely expressed in a stage-specific manner (5), and TRAP itself is expressed by sporozoites. TRAP is considered a high-priority subunit malaria vaccine candidate because it is exposed at the sporozoite surface and because genetic deletion of trap in *Plasmodium berghei* showed it is essential for motility and invasion (6). A virally vectored TRAP-based vaccine is able to mediate protective effects in both animal infection models and humans (7), making a more-detailed understanding of TRAP function a research priority to improve these vaccines and expand our basic knowledge of parasite motility and invasion.

TRAP is a typical I cell surface protein containing both a von Willebrand factor A (VWA) and a thrombospondin type 1 repeat (TSR) domain. VWA and TSR domains are found in mammalian proteins such as integrins and complement factors, where they bind extracellular ligands, suggesting a similar role in TRAP. This is supported by genetic studies showing that mutation of the VWA and TSR domains does not affect sporozoite motility but significantly impairs host cell invasion (8) by the presence of an integrin-like metal ion-dependent adhesion site (MIDAS) in the TRAP ectodomain (8), and by the binding of recombinant proteins corresponding to the TRAP extracellular region to human hepatocyte-derived cell lines (9, 10). Structural

Significance

Malaria is caused by a parasite that is deposited in the skin through the bite of an infected mosquito. From the skin, parasites navigate through host tissues where they must locate and invade liver cells. We know that a parasite surface protein called TRAP is important for this process, making it a leading vaccine candidate. TRAP is thought to work by specifically binding a defined host cell surface protein, but its identity has remained a long-standing mystery. Our research has identified an integrin—a class of host cell surface proteins—as a TRAP receptor. This finding provides an important piece of the puzzle relating to TRAP function and may help improve the development of an effective malaria vaccine.

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domains which open into an elongated shape, providing the force to trigger a conformational change in the tandem VWA and TSR domains. Recombinant studies have suggested that extracellular binding events may challenge due to the difficulties in solubilizing membrane-embedded proteins in their native conformation and the often transient nature of their interactions (13). We have developed approaches to express the ectodomains of receptor proteins from Plasmodium falciparum in a functionally active form (14) and used them in a protein interaction assay called AVEXIS (avidity-based extracellular interaction screen) (15) that circumvents some of these challenges. Here, we show how we have used this approach to identify the human integrin αvβ3 as a host receptor for P. falciparum TRAP.

Results

P. falciparum TRAP Expressed in HEK293 Cells Binds HepG2 Cells Independently of Cell Surface Sulfates. To identify human host receptors for P. falciparum TRAP, we expressed the entire ectodomain as a soluble recombinant protein in mammalian cells. Because interactions between cell surface receptor proteins are typically weak, we purposefully oligomerized monomeric TRAP around a streptavidin–phycoerythrin conjugate to form a fluorescent highly avid binding probe which stained the surface of human HepG2 cells (Fig. 1A). To determine if this cell binding was due to cell surface sulfated glycoconjugates, we tested whether the TRAP ectodomain could interact with heparin, chondroitin sulfate A, or heparan sulfate using a sensitive surface plasmon resonance assay. Heparin, chondroitin sulfate, and heparan sulfate were injected at 1 mg/mL for 60 s over biotinylated TRAP immobilized on a streptavidin-coated sensor chip, with no observable binding. (C) TRAP binding to HepG2 cells is unaffected by an excess of sulfated glycoconjugates. The binding of TRAP to HepG2 cells was unaffected when preincubated with 250 μg/mL heparin, 250 μg/mL chondroitin sulfate, or 100 μg/mL heparan sulfate. (D) TRAP binds HepG2 cells independently of cell surface sulfated glycans. HepG2 cells in which the SLC35B2 gene had been targeted using CRISPR/Cas9 technology retained TRAP binding. Negative controls in A, B, and D show that Cd200 protein, expressed and oligomerized in the same way as TRAP, does not bind HepG2 cells. -ve, negative.

An important question is the identity of the extracellular molecules displayed on host cells that can interact with TRAP and how these interactions are involved in the pathogenesis of malaria. Previous work has suggested that TRAP interacts with sulfated glycoconjugates (9), but the significance of this is not clear (10). Identifying extracellular interactions between cell surface receptor proteins can be challenging due to the difficulties in solubilizing membrane-embedded proteins in their native conformation and the often transient nature of their interactions (13). We have developed approaches to express the ectodomains of receptor proteins from Plasmodium falciparum in a functionally active form (14) and used them in a protein interaction assay called AVEXIS (avidity-based extracellular interaction screen) (15) that circumvents some of these challenges. Here, we show how we have used this approach to identify the human integrin αvβ3 as a host receptor for P. falciparum TRAP.

Fig. 1. Recombinant P. falciparum TRAP binds HepG2 cell surfaces independently of sulfated glycoconjugates. (A) A highly avid fluorescent P. falciparum TRAP binding reagent stained the surface of HepG2 cells. Monomeric biotinylated TRAP ectodomain was clustered around a streptavidin–phycoerythrin (PE) conjugate and bound HepG2 cells, relative to a Cd200 negative control. (B) TRAP did not interact with sulfated glycoconjugates in surface plasmon resonance assays. Heparin, chondroitin sulfate, and heparan sulfate were injected at 1 mg/mL for 60 s over biotinylated TRAP immobilized on a streptavidin-coated sensor chip, with no observable binding. (C) TRAP binding to HepG2 cells is unaffected by an excess of sulfated glycoconjugates. The binding of TRAP to HepG2 cells was unaffected when preincubated with 250 μg/mL heparin, 250 μg/mL chondroitin sulfate, or 100 μg/mL heparan sulfate. (D) TRAP binds HepG2 cells independently of cell surface sulfated glycans. HepG2 cells in which the SLC35B2 gene had been targeted using CRISPR/Cas9 technology retained TRAP binding. Negative controls in A, B, and D show that Cd200 protein, expressed and oligomerized in the same way as TRAP, does not bind HepG2 cells. -ve, negative.

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Fig. 2. Integrin αvβ3 is a human receptor for P. falciparum TRAP. (A) P. falciparum TRAP ectodomain expressed as a prey was tested for binding using the AVEXIS assay against a library of 65 human receptor ectodomains from the indicated architectural classes; bait numbers correspond to named proteins in SI Appendix, Table S1. Bars represent mean ± SD, n = 2. (B) Biotinylated integrin αvβ3 was clustered around streptavidin–alkaline phosphatase to create an avid binding reagent and was captured by an immobilized monomeric TRAP (black circles), but not control Cd200 (blue squares). Biotinylated Cd200 similarly clustered around streptavidin–alkaline phosphate did not interact with immobilized TRAP (purple triangles). Data points represent mean ± SD, n = 3. Data shown are representative of at least three independent experiments. (C) Kinetic analysis showed that TRAP and integrin αvβ3 directly interact. Increasing concentrations of purified TRAP ectodomain were injected at a high flow rate (100 μL min⁻¹) for a contact time of 20 s over biotinylated integrin αvβ3 immobilized on a streptavidin-coated chip; binding was quantified relative to a reference flow cell.
of glycoalyx components, including heparan sulfate. We demonstrated that TRAP binding to SLC35B2-targeted cells was largely unaffected (Fig. 1D), even though we could show that a significant population of these cells had lost FGFR1 binding, a known heparan sulfate binding protein (SI Appendix, Fig. S1). These data demonstrate that recombinant TRAP expressed in mammalian cells interacted with a cell surface receptor expressed on HepG2 cells distinct from cell surface sulfated glycans.

The Integrin αβ3 Is a Human Receptor for P. falciparum TRAP. To determine the molecular identity of the TRAP receptor on HepG2 cells, we selected a panel of 65 proteins from an existing library of human cell surface receptor ectodomains available in the laboratory (16) that were expressed on HepG2 cells (17) (SI Appendix, Table S1). This array of human receptors was probed for direct interactions with TRAP using the AVEXIS assay, and a clear interaction with integrin αβ3 was immediately observed (Fig. 2A). Integrins are a family of cell surface receptors consisting of a complex of two noncovalently associated polypeptide chains whose extracellular regions can exist in different ligand affinity states formed by conformational changes induced by intracellular or extracellular signaling events (18). We demonstrated that this interaction was independent of the “bait–prey” orientation by clustering the biotinylated αβ3 integrin around a streptavidin-alkaline phosphatase conjugate and showing that it interacted robustly with TRAP, but not with a control bait (Fig. 2B). We next demonstrated that TRAP and αβ3 interacted directly using surface plasmon resonance, by injecting serial dilutions of purified TRAP over a surface containing immobilized integrin αβ3. Kinetic analysis showed that the two proteins interacted directly, from which association and dissociation rate constants of 2.95 ± 0.02 × 10^7 M^-1 s^-1 and 0.02 ± 0.01 s^-1 were calculated, corresponding to an interaction half-life of over 3 min (Fig. 2C). This binding affinity was unexpectedly high for an extracellular receptor–ligand interaction, which often have half-lives in the order of seconds (13). Together, these data indicate that human αβ3 interacts directly with P. falciparum TRAP.

The RGD Motif in TRAP Is Necessary, but Not Sufficient, for Binding Integrin αβ3. A well-characterized ligand binding determinant for a subset of mammalian integrins, including αβ3, is a three-amino-acid motif composed of arginine-glycine-aspartic acid (RGD) (19). P. falciparum TRAP contains an RGD sequence between the TSR domain and the predicted transmembrane-spanning region. To determine the importance of this sequence in integrin binding, we first added a cyclized peptide containing an RGD motif, which is an inhibitor of RGD-dependent αβ3 interactions (20). Serial dilutions of the cyclo(RGDfV) peptide showed a dose-dependent inhibition of the interaction (Fig. 3A). To confirm the importance of this motif, we used site-directed mutagenesis to mutate the RGD sequence in TRAP to RGE, a conservative mutation known to disrupt ligand binding. We observed that the mutant RGE TRAP was unable to bind human αβ3 (Fig. 3B), confirming that the RGD sequence was necessary for the interaction. The RGD sequence is not a conserved feature of orthologous TRAP protein sequences from other Plasmodium species. To determine if the interaction was conserved across species, the entire extracellular domains of RGD-containing TRAP orthologs from two human parasites (Plasmodium vivax and Plasmodium knowlesi) and non RGD-containing TRAP orthologs from two rodent parasites (Plasmodium berghei) were expressed and tested for their ability to interact with human integrin αβ3. None of the TRAP orthologs that lacked the RGD motif bound, confirming the importance of this motif in binding αβ3 (Fig. 3C). While P. praefaliciparum bound equivalently to P. falciparum, surprisingly, no binding of the RGD-containing P. reichenowi TRAP was observed, suggesting the requirement for additional integrin αβ3 binding determinants (Fig. 3C). Consistent with this, introducing an RGD motif at an equivalent position in P. vivax TRAP did not result in a gain of integrin αβ3 binding activity (Fig. 3C). Together, these data indicate that while the TRAP RGD motif is necessary, it is not sufficient for the interaction with integrin αβ3.

The VWA Domain of P. falciparum TRAP Is Required for Integrin αβ3 Binding. To determine which other regions of TRAP are involved in the interaction, we expressed discrete domains of the TRAP ectodomain and tested them for αβ3 binding. We could again show that the RGD sequence was insufficient for binding, since a protein containing the TSR domain and the RGD-containing repeat region up to the transmembrane region did not interact with αβ3 (Fig. 4A). This also suggested that the TSR domain was not involved in the interaction, and this was supported by the fact that the TSR domain alone did not interact (Fig. 4A). These data implied that the VWA domain might encode the additional binding determinant, and although we were unable to express the
much reduced binding to αvβ3 in the AVEXIS assay (Fig. 4C). Consistent with this, titrating the divalent cation chelator EDTA into a TRAP binding assay showed a dose-dependent inhibition of binding (Fig. 4D). Together, these data suggest that TRAP interacts with αvβ3 at two sites, with contributions from both the VWA domain involving the MIDAS, and the RGD sequence.

**HepG2 Cells Targeted for ITGAV Lose All TRAP Binding Activity.** We returned to cell-based assays to investigate the αvβ3-TRAP interaction within the context of an intact cell. We observed that the cell surface binding of TRAP was significantly increased in the presence of manganese ions, which promote the formation of proadhesive active integrin conformations (21), consistent with a role for integrins in their active conformation in TRAP binding (Fig. 5A). Preincubating cells with an inhibitory αvβ3 monoclonal antibody whose epitope lies within the αv-subunit reduced TRAP binding (Fig. 5B). Although this incomplete blocking is consistent with TRAP using more than one contact site on αvβ3, it could also indicate the presence of additional receptors on HepG2 cells. To establish this, we again used CRISPR/Cas9 technology to genetically target the genes encoding both the ITGAV and ITGB3 genes in HepG2 cells (SI Appendix, Fig. S2). Strikingly, in ITGAV-targeted cells, all TRAP binding was lost, demonstrating that the αv-subunit is the major determinant of TRAP binding (Fig. 5C). Cells in which the ITGB3 gene had been targeted, however, did not show any loss in cell surface TRAP binding (Fig. 5C). Despite repeated attempts, the HepG2 cell lines were refractory to single-cell cloning, so we used a human erythroleukemia (HEL) cell line which we knew could be made clonal and had high expression levels of both αv and β3-subunits. Similar to HepG2, all TRAP binding was lost in ITGAV-targeted HEL cells, whereas targeting of ITGB3 had no

Fig. 4. The *P. falciparum* TRAP VWA domain is required for integrin αvβ3 binding. (A, Upper) A series of truncations of the *P. falciparum* TRAP extracellular domain were expressed as pentameric preys. C-Term, C terminus; SP, signal peptide; TM, transmembrane. (Lower) Binding with integrin αvβ3 quantified by AVEXIS. The VWA domain alone was expressed at insufficient levels for use in the assay. Only the full-length extracellular domain of TRAP was able to fully interact with αvβ3 bait in AVEXIS; however, residual binding signal was observed for the VWA + TSR construct. (B, Upper) Replacing the VWA domain in the nonbinding *P. reichenowi* TRAP with the orthologous domain from *P. falciparum* was sufficient to confer the ability to bind integrin αvβ3. The entire extracellular regions of *P. falciparum* (P) and *P. reichenowi* (Pr) TRAP, as well as a VWA domain replacement chimera were expressed as prey proteins. (Lower) Binding activity to integrin αvβ3 quantified by AVEXIS. (C) Mutating two conserved serine residues within the MIDAS sequence of the *P. falciparum* TRAP VWA domain significantly decreased binding to αvβ3. TRAP and the S56A S58A TRAP were expressed as preys and used in the AVEXIS assay to test binding against αvβ3 bait. In (A–C), the positive control (+ve) is the Cd200–Cd200R interaction and the negative control (−ve) was Cd200 bait and TRAP prey. Bars represent means ± SD, n = 3. A representative experiment of at least two independent experiments is shown. (D) The TRAP–αvβ3 interaction is divalent cation dependent. Dose-dependent inhibition of the TRAP–αvβ3 interaction, but not the Cd200–Cd200R interaction, was observed using the AVEXIS assay when prey proteins were preincubated with increasing concentrations of divalent cation chelator EDTA. Data points represent mean ± SD, n = 3. A representative experiment of at least three independent experiments is shown.

VWA domain in isolation, a protein containing both VWA and TSR domains but lacking the RGD sequence showed weak residual binding to αvβ3 (Fig. 4A). To examine the role of the VWA domain in the context of the entire extracellular region, we constructed a chimeric TRAP protein in which the VWA domain was replaced by the Cd200 domain from *P. falciparum* (Fig. 4B). This chimeric TRAP protein was able to interact with αvβ3 in the AVEXIS assay (Fig. 4B), suggesting that the minimum requirements for the αvβ3 interaction are the *P. falciparum* VWA domain and RGD sequence. To investigate this further, we mutated conserved residues in the MIDAS sequence located within the VWA domain that are required for coordinating divalent cations important for ligand binding. A protein containing the double mutation S56A S58A displayed...
cells. Unlike the parental HepG2 cell line, we observed that our ITGAV-targeted HepG2 cells grew in small clusters that adhered poorly to tissue culture plastic; however, precoating the substrate with collagen improved cellular adherence to a degree that sporozoite invasion experiments could be attempted. No significant difference in the ability of P. falciparum sporozoites to invade ITGAV-targeted compared with parental HepG2 cells was observed (Fig. 6D), although we did not consider this conclusive due to the altered adhesive properties of the cells. To circumvent these difficulties, we added anti-αv antibodies (Fig. 6B) and soluble integrin ectodomains (Fig. 6C) to P. falciparum sporozoite invasion assays and, again, observed no strong effect, suggesting that this interaction has no major role in sporozoite invasion of hepatocytes. Sporozoites interact with many different tissues before invading hepatocytes. Because we observed that P. falciparum TRAP was able to interact with the mouse αvβ3 ortholog (SI Appendix, Fig. S34), we genetically replaced P. berghei TRAP with either the P. falciparum ortholog or a nonbinding RGE mutant. Although many functions of P. berghei TRAP were complemented, we repeatedly observed that the transgenic parasites did not glide or develop in a hepatocyte cell line compared with control parental P. berghei parasites, suggesting that some aspects of TRAP function were not fully complemented, precluding confident interpretations of the role of the interaction using this approach (SI Appendix, Fig. S33). To investigate the requirement of the interaction in the host, we next infected Ifgβ3-deficient mice with fluorescent transgenic P. falciparum parasites and tracked their motility and behavior in the dermis using intravital microscopy (22). We observed that P. falciparum sporozoites glide with greater speed (Fig. 6D) and displace over larger distances from the inoculation site in Ifgβ3-deficient mice compared with wild-type siblings (Fig. 6E). Together, these data suggest that the TRAP-integrin interaction has no major role in P. falciparum hepatocyte invasion but influences the behavior of sporozoites in the dermis.

Discussion

In contrast to the detailed descriptions of how sporozoites interact with host cells, remarkably little is known about the molecular interactions involved. Both circumsporozoite protein (CSP) and TRAP have been demonstrated to interact with highly sulfated proteoglycans displayed on tissues in the liver sinusoids, which may provide a molecular explanation for sporozoite homing to this organ (23, 24). Other cell surface molecules that have roles in the liver stage of infection include CD81, SR-B1, CD68, and EphA2, but the details of other cell surface molecules that have roles in the liver stage of infection include CD81, SR-B1, CD68, and EphA2, but the details of how the parasite interacts with the hepatocyte to initiate invasion and cytokinesis are not fully understood. It is likely that in vivo, TRAP is able to interact with human αvβ3-subunit-containing integrins provides an important precedent demonstrating that sporozoite surface proteins do interact with host cell surface receptor proteins. Here, we have shown a critical requirement for the integrin αvβ3-subunit in TRAP binding at cell surfaces, but because this integrin αvβ3-subunit is expressed in most tissues, the expression pattern of the β3-subunit partners may determine cellular binding selectivity. Our experiments suggest redundancy in β3-subunit usage, with both β5 and β6 expressed, whereas β3 is restricted, with the αvβ3 integrin expressed highly on osteoclasts but also on endothelial cells, vascular smooth muscle, and platelets (25). Although these integrins are broadly expressed, we have shown that TRAP binding is promoted in the presence of manganese ions that are proadhesives integrin “activators,” suggesting that TRAP preferentially binds integrins in their activated ligand binding conformation. A preference for binding integrins in their active conformation would restrict the number of receptors available and may serve to localize the TRAP-integrin interactions to clusters of integrins in their activated state, as has been suggested for focal adhesions (26). The sporozoite may exploit these binding preferences as a signal to switch between gliding and cell traversal or invasion behaviors. Using in vitro HepG2 sporozoite invasion assays, however, we did not observe any major effect when perturbing the interaction.

The TRAP-Integrin Interaction Has No Major Role in Hepatocyte Invasion, but May Be Involved in Dermal Migration. To investigate the functional role of the TRAP-integrin interaction, we first used in vitro P. falciparum sporozoite invasion assays of HepG2

Fig. 6. The TRAP-integrin interaction has no major role in hepatocyte invasion but may be involved in sporozoite dermal migration. The effect of perturbing the TRAP-integrin interaction on the ability of P. falciparum sporozoites to invade HepG2 cells in vitro was assessed by comparing ITGAV-targeted cells relative to parental HepG2-Cas9 controls (A); by allowing sporozoites to invade in the continued presence of the anti-αv monoclonal antibody (100 μg/mL) or an isotype-matched control antibody (100 μg/mL) (B); or by comparing 200 nM TRAP binding soluble recombinant integrin ectodomain αvβ3 relative to nonbinding control αvβ1 (C). Bars represent mean ± SD, n = 2. (D) Itgb3−/− C57BL/6 mice and wild-type (WT) siblings were infected with fluorescent P. falciparum sporozoites by intradermal injection into the ear pinna, and their circular motility was observed by intravital video microscopy at 5 min (Itgb3−/−, 260 tracks, n = 3; WT, 316 tracks, n = 3) and 10 min (Itgb3−/−, 297 tracks, n = 5; WT, 338 tracks, n = 5). Speed data are represented as box and whisker plots, with the horizontal line representing the mean and the whiskers representing the 10th to 90th percentiles; outliers are plotted individually. (E) Displacement of P. falciparum sporozoites 5 and 10 min after injection, represented as box and whisker plots, with the horizontal line representing the mean and the whiskers representing the 10th to 90th percentiles. ****P ≤ 0.0001 Kruskal-Wallis test. p.i., postinjection.

The TRAP-Integrin Interaction Has No Major Role in Hepatocyte Invasion, but May Be Involved in Dermal Migration. To investigate the functional role of the TRAP-integrin interaction, we first used in vitro P. falciparum sporozoite invasion assays of HepG2

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using antibodies, recombinant proteins, or cells lacking the integrin αv-β3 subunit. To investigate the possibility that the interaction may occur at a different stage of the sporozoite journey from the dermis to the hepatocytes, we used intravital microscopy to quantify sporozoite behavior in the dermis of transgenic mice. Igαv-deficient mice are embryonic lethal (27), and although functional redundancies within integrin β-chain are a possibility, we observed that sporozoites were able to move faster within the dermis of Igαv-deficient mice, suggesting that the interaction could impede the movement of the parasites. This would suggest that TRAP does not use integrin binding as a gliding substrate, but rather as a stop or brake to alter its gliding behavior in response to a localized cellular signal in the host. The VWA domain in TRAP is structurally homologous to the I-domain of integrins, and structural studies have shown that both TRAP VWA and the integrin I-domain contain metal ion-dependent adhesion sites whose affinity for ligand can be regulated through conformational changes and thereby regulate integrin adhesiveness and, potentially, the extension of the TRAP ectodomain (11, 28). Analysis of the cocrystal structure of the RGD-containing pro-TGF-β ligand with the αvβ6 integrin has revealed that upon ligand binding, integrins can apply force to and thereby reshape their ligands (29). Pro-TGF-β1 binds its integrin receptor with a high affinity (K_D of ~50 nM) (30), similar to the affinity of TRAP for its integrin receptor (K_D of ~10 nM). One possibility is that the high affinity of TRAP binding and conformational changes in the integrin results in the application of force through TRAP, which influences parasite motility by locking the extracellular regions of TRAP into its extended conformation.

Since the demonstration over 20 y ago that TRAP is essential for parasite motility, it has been a focus for malaria vaccines targeting the precytoytic stage of Plasmodium infections. By identifying αv-β3-containing integrins as a host receptor for TRAP, we have addressed one of the main outstanding questions relating to the function of TRAP and provide an important example that sporozoite ligands can directly interact with host receptors.

Materials and Methods

Full details are available in SI Appendix, SI Materials and Methods.

Recombinant Protein Expression and Protein Interaction Assays.

Proteins were expressed in HEK293 cells and AVEKIS assays were performed as previously described (15). In binding assays requiring EDTA or cyclo(RGDfV), serial dilutions were preincubated with the prey proteins before addition to the baits. Avid integrin preys were made by clustering bivalent integrin heterodimers around a streptavidin-alkaline phosphatase conjugate. Cell binding was performed by clustering bivalent proteins around a fluorescent streptavidin conjugate before presenting to cells and analysis by flow cytometry.

Gene Targeting Using CRISPR/Cas9.

Gene-specific guide RNAs were delivered to Cas9-expressing cell lines by lentiviral transduction after cloning guide RNAs into the pKLV2-UsgRNA-PGKpuro2A-1BP-W expression vector. Gene-targeted cells were selected using puromycin and were cultured for at least 8 d before use.

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