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Crystal Structure of *Plasmodium knowlesi* Apical Membrane Antigen 1 and Its Complex with an Invasion-Inhibitory Monoclonal Antibody

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Abstract

The malaria parasite *Plasmodium knowlesi*, previously associated only with infection of macaques, is now known to infect humans as well and has become a significant public health problem in Southeast Asia. This species should therefore be targeted in vaccine and therapeutic strategies against human malaria. Apical Membrane Antigen 1 (AMA1), which plays a role in *Plasmodium* merozoite invasion of the erythrocyte, is currently being pursued in human vaccine trials against *P. falciparum*. Recent vaccine trials in macaques using the *P. knowlesi* orthologue PkAMA1 have shown that it protects against infection by this parasite species and thus should be developed for human vaccination as well. Here, we present the crystal structure of Domains 1 and 2 of the PkAMA1 ectodomain, and of its complex with the invasion-inhibitory monoclonal antibody R31C2. The Domain 2 (D2) loop, which is displaced upon binding the Rhoptry Neck Protein 2 (RON2) receptor, makes significant contacts with the antibody. R31C2 inhibits binding of the Rhoptry Neck Protein 2 (RON2) receptor by steric blocking of the hydrophobic groove and by preventing the displacement of the D2 loop which is essential for exposing the complete binding site on AMA1. R31C2 recognizes a non-polymorphic epitope and should thus be cross-strain reactive. PkAMA1 is much less polymorphic than the *P. falciparum* and *P. vivax* orthologues. Unlike these two latter species, there are no polymorphic sites close to the RON2-binding site of PkAMA1, suggesting that *P. knowlesi* has not developed a mechanism of immune escape from the host's humoral response to AMA1.

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Introduction

Human malaria was long thought to be restricted to infection by four *Plasmodium* species: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. It has now been confirmed, however, that natural human infection also occurs with *P. knowlesi* [1], a species hitherto associated only with macaque hosts. Human infection by *P. knowlesi*, previously confused with infection by the less virulent *P. malariae*, is quite widespread in Southeast Asia and can lead to mortality [2–4]. Accordingly, there is a need to include *P. knowlesi* in therapeutic and vaccine strategies against human malaria.

Apical Membrane Antigen 1 (AMA1), a type 1 transmembrane protein of the *Plasmodium* parasite, includes an ectodomain, a transmembrane region and a cytoplasmic domain. The ectodomain comprises three domains referred to as Domain 1, Domain 2 and Domain 3. AMA1 is produced in the microneme organelles and transferred to the parasite surface just prior to or during red blood cell (RBC) invasion [5]. First detected in *P. knowlesi* [6], AMA1 was later found in other *Plasmodium* species, as well as in other members of the *Apicomplexa* phylum [7–9]. AMA1 appears to be essential for invasion since, for several *Plasmodium* species, antibodies raised against the ectoplasmic region of the protein have been shown to inhibit invasion, and immunization with AMA1 in animal models protects against infection [10–14]. In spite of significant polymorphism, it is a leading malaria vaccine candidate and vaccine formulations based on the *P. falciparum* AMA1 ectodomain are currently being pursued in clinical trials [15, 16].

Crystal structures of AMA1 from *Plasmodium* species and other members of the *Apicomplexa* phylum (*P. vivax* [17], *P. falciparum* [18], *Toxoplasma gondii* [19], *Babesia babesia* [20] and *Neospora caninum* [20]) have revealed the presence of a hydrophobic groove on Domain 1 of the protein. This region is targeted by invasion-inhibitory monoclonal antibodies [21, 22], suggesting that it forms a receptor-binding site. The receptor for AMA1 is the Rhoptry Neck Protein (RON) complex, which is transferred from the rhoptries to the host cell membrane during invasion [23, 24]. In particular, it has been shown in *T. gondii* and *P. falciparum* that AMA1 interacts directly with the component RON2 of the receptor [25,26]. Furthermore, crystal structures of the complex formed between TgAMA1 or PfAMA1 and a peptide derived from the extracellular region of RON2 from each of these respective species have confirmed that the hydrophobic groove on AMA1 contributes to the receptor-binding site [27, 28]. Moreover, these studies showed that, in addition to the hydrophobic groove, an adjacent surface that becomes exposed upon displacement of a flexible region known as the Domain 2 (D2 loop) also contributes to the RON2-binding site. The AMA1-RON interaction appears to take place at the tight junction, which forms between the merozoite and RBC membranes as the parasite enters the host cell and is a critical component in the invasion process [29]. This model has been subject to controversy, however, with arguments for and against [30–33], showing that further experimental analysis is required to clarify this issue.

The monoclonal antibody (mAb) R31C2, raised in rats against the W1 variant of *P. knowlesi* merozoites, is specific for *Plasmodium knowlesi* AMA1 (PkAMA1) and inhibits *in vitro* multiplication of the parasite [6]. R31C2 was the first anti-AMA1 mAb to be characterized (along with mAb R32C3) and has proved to be a useful tool in dissecting the role of AMA1 in RBC infection. Since its Fab fragment is also a highly effective inhibitor, it was concluded that the mAb acts by blocking a receptor-binding site on PkAMA1 [34]. Electron microscopy studies of *P. knowlesi* merozoites in the presence of R31C2 have shown that the parasite makes extensive contacts with the RBC surface, characteristic of the random attachment that occurs during the first stage of invasion [35]. However, no apical attachment to the RBC surface nor the subsequent formation of a tight junction between the merozoite and RBC membranes—the ensuing