Anti-CD81 but not anti-SR-BI blocks Plasmodium falciparum liver infection in a humanized mouse model

Lander Foquet1, Cornelus C. Hermsen2, Lieven Verhoye1, Geert-Jan van Gemert2, Riccardo Cortese3, Alfredo Nicosia4,5, Robert W. Sauerwein2, Geert Leroux-Roels1 and Philip Meuleman1*

1Center for Vaccinology, Ghent University, De Pintelaan 185, 9000 Gent, Belgium; 2Radboud University Medical Center, Geert Grooteplein 28, 6525 GA Nijmegen, The Netherlands; 3Keires AG, Elisabethenstrasse 15, 4051 Basel, Switzerland; 4CEINGE, Via Comunale Margherita, 484-538, 80131 Naples, Italy; 5Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Corso Umberto I, 80138 Naples, Italy

*Corresponding author. Tel: +32-9-332-02-05; Fax: +32-9-332-63-11; E-mail: philip.meuleman@ugent.be

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Objectives: Plasmodium falciparum sporozoites, deposited in the skin by infected Anopheles mosquitoes taking a blood meal, cross the endothelium of skin capillaries and travel to the liver where they traverse Kupffer cells and hepatocytes to finally invade a small number of the latter. In hepatocytes, sporozoites replicate, differentiate and give rise to large numbers of merozoites that are released into the bloodstream where they invade red blood cells, thus initiating the symptomatic blood stage. Using in vitro systems and rodent models, it has been shown that the hepatocyte receptors CD81 and scavenger receptor type B class I (SR-BI) play a pivotal role during sporozoite invasion. We wanted to evaluate whether these two entry factors are genuine drug targets for the prevention of P. falciparum infection in humans.

Methods: Immunodeficient mice of which the liver is largely repopulated by human hepatocytes were treated with monoclonal antibodies blocking either CD81 or SR-BI 1 day prior to challenge with infected mosquitoes. P. falciparum infection of the liver was demonstrated using a qPCR assay.

Results: In human liver chimeric mice, an antibody directed against CD81 completely blocked P. falciparum sporozoite invasion while SR-BI-specific monoclonal antibodies did not influence in vivo infection.

Conclusions: These observations confirm the role of CD81 in liver-stage malaria and question that of SR-BI. CD81 might be a valuable drug target for the prevention of malaria.

Keywords: malaria, P. falciparum, sporozoites, liver stage, CD81, SR-BI, hepatocyte entry

Introduction

In 2010, Plasmodium falciparum was responsible for 200 million cases of malaria and 650 000 deaths. The lack of an effective vaccine and increasing spread of drug-resistant parasites have a devastating impact on global health, especially in sub-Saharan Africa.1

During a blood meal, infected Anopheles mosquitoes inject 10–100 sporozoites into the skin.2 These parasites enter the bloodstream and travel to the liver where they traverse Kupffer cells and hepatocytes to finally invade a small number of hepatocytes.2 Plasmodium sporozoites can enter host cells by two distinct pathways. Parasites either disrupt the plasma membrane followed by a ‘traversal’ migration. Alternatively, they can form an ‘invasion’ vacuole.3 While ‘traversal’ lacks cell selectivity, ‘invasion’ is a restricted process. A series of still poorly understood interactions between the parasite and the host cell leads to the formation of a parasite compartment surrounded by a host-derived membrane inside the hepatocyte, called the parasitophorous vacuole (PV), wherein the parasite resides and further develops. P. falciparum exclusively infects humans and a few non-human primate species.1 This narrow host tropism is determined by specific surface receptors on the hepatocyte membrane that are essential for parasite entry and intracellular host molecules required for growth and development. Silvie et al.4,5 have shown that the tetraspanin CD81 is required for in vitro hepatocyte infection by P. falciparum sporozoites. However, transgenic expression of CD81 did not render HepG2 cells permissive to infection by P. falciparum,6 suggesting that other molecules, located on the surface or inside hepatocytes, are necessary to enable invasion and PV formation. Experimental evidence has been provided indicating a possible role for scavenger receptor class B type I (SR-BI)
in hepatocyte invasion and infection by P. falciparum in vitro as well as in vivo for the rodent parasites Plasmodium berghei and Plasmodium yoelii.6 SR-BI inhibition in primary human hepatocytes (huHEPs) with the chemical compound BLT-1 caused a significant and specific decrease in P. falciparum infection.6 Using SR-BI-knockout, SR-BI-hypomorphic and SR-BI-transgenic primary hepatocytes, as well as SR-BI-specific antibodies, Rodrigues et al.6 showed that SR-BI significantly increased hepatocyte permisiveness to P. falciparum, P. yoelii and P. berghei entry and promoted parasite maturation within the hepatocyte.

CD81 and SR-BI have also been recognized as essential (co)receptors for hepatitis C virus (HCV) entry into huHEPs.8 Using uPA-transgenic SCID mice of which the liver is repopulated with primary huHEPs (humanized mice)9 we have demonstrated that a blockade of CD81 or SR-BI with monoclonal antibodies (MAbs) completely prevented HCV infection in vivo.10–13 We and others have shown that humanized mice can also be infected with P. falciparum.14–16 In this study, we investigated the effect on in vivo hepatocyte infection of the administration of anti-CD81 and anti-SR-BI MAbs to humanized mice prior to infection with P. falciparum via bites of infected mosquitoes.

**Materials and methods**

**Generation of humanized mice**

Humanized uPA+/u–SCID mice were generated as described previously.9 Only animals with human albumin levels >2 mg/mL were used. All animal experiments were approved by the Animal Ethics Committee of the Faculty of Medicine and Health Sciences of Ghent University and were conducted in accordance with Belgian and European legislation.

**MAbs**

Anti-CD81 (JS-81, mouse IgG1) was purchased from BD Biosciences (San Jose, CA, USA). The SR-BI-specific MAb1671 and MAb C11 were generated as described previously.17

**In vivo parasite challenge and prophylactic treatment experiments**

One day prior to mosquito bite challenge, 400 μg of MAbs targeting CD81 (JS-81) or SR-BI (MAB1671) was injected intraperitoneally (ip) into humanized mice.10–13 Control animals were injected with an equal volume of PBS. Using SR-BI-knockout mice, it was shown that SR-BI significantly increases hepatocyte permisiveness to P. falciparum, P. yoelii and P. berghei entry and promotes parasite maturation within the hepatocyte.

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**Isolation and detection of P. falciparum DNA and huHEP DNA by qPCR**

Five days after infection, mice were euthanized and their livers were analyzed as described previously.14 Briefly, each liver was divided into 12 sections. DNA was extracted from 25 mg of tissue with the High Pure PCR Template Preparation Kit (Roche). P. falciparum DNA levels were quantified using a highly sensitive qPCR assay. qPCR was also employed to assess the degree of repopulation with huHEPs and to normalize P. falciparum copy numbers.

**Statistics**

For statistical analysis, the number of parasites per 10⁶ huHEPs was determined in 12 different liver samples per mouse and all data obtained from the different mice were combined per treatment group. The Kruskal–Wallis test (non-parametric ANOVA), followed by Dunn’s multiple comparisons test, was used to determine whether the difference between three treatment groups (control versus MAB JS-81 versus MAB1671) was statistically significant. The Mann–Whitney U-test was used to evaluate a potential significant difference between two treatment groups (control versus MAB C11). All statistical analyses were calculated using GraphPad Prism software.

**Results and discussion**

Prevention of malaria is mainly based on drugs that target the erythrocytic stage of the parasite life cycle.5 Due to the increasing occurrence of drug resistance, there is an urgent need for new prophylactic and curative strategies. The pre-erythrocytic stage appears to be an ideal target since intervention at this level can prevent both infection and transmission.5 In other infectious diseases, small molecule inhibitors have proven to be highly effective as entry inhibitors. The chemokine receptor antagonist maraviroc is currently used to prevent cell entry by HIV-1 in infected patients.18 Targeting the highly conserved host cell protein CCR5, rather than the virus, circumvents the problems related to the high variability of HIV-1 and the occurrence of escape variants. Identification of hepatocyte-specific membrane molecules necessary for sporozoite entry and invasion could lead to receptor antagonists that block malaria’s liver-stage infection.

Administration of a single 400 μg dose of the anti-CD81 MAB JS-81 1 day before exposure to infected mosquitoes completely prevented P. falciparum infection in humanized mice. While P. falciparum DNA was undetectable in the liver of treated mice (<0.75 parasites/10⁶ huHEPs), the control mice had a mean liver parasite burden of 633.7 parasites/10⁶ huHEPs (Figure 1). The same antibody was previously shown to prevent HCV infection in this model.10 Our observation provides in vivo confirmation of the previous finding that CD81 is an essential host factor for P. falciparum infection of human hepatocytes in vitro.15,16

Next, we examined the capacity of anti-SR-BI therapy to prevent P. falciparum infection. The administration of a single 400 μg dose of MAB1671 did not protect humanized mice from infection (Figure 1). Moreover, the liver parasite burden measured 5 days after mosquito bite infection (1387.2 parasites/10⁶ huHEPs) was not different from that of untreated control mice (633.7 parasites/10⁶ huHEPs). To rule out that this lack of activity was caused by the characteristics of the MAB used (MAB1671), the in vivo experiment was repeated using the SR-BI-specific MAB C11 that had been successfully used in the in vitro study that had inspired us.7 In addition, two doses of 400 μg of C11 were given, 1 day before and 1 day after the mosquito challenge. These changes to the protocol had no influence on the experimental outcome since all mice became infected and again no difference was observed in liver parasite burden between treated (315.8 parasites/10⁶ huHEPs) and control mice (252.0 parasites/10⁶ huHEPs) (Figure 2). Since MAB1671 has been shown to prevent HCV entry in vitro and in vivo11 and since C11 was originally used...
More work is needed to further unravel *P. falciparum* sporozoite invasion and development in an infected hepatocyte. It is still not clear with which liver cell membrane molecules *P. falciparum* sporozoites directly interact. In addition, not only the presence but also the absence of a membrane molecule may determine cellular tropism. For example, it has been demonstrated that cells expressing both CD81 and EWI-2wint are resistant to HCV infection, whereas hepatocytes that only express CD81 and no EWI-2wint are susceptible to HCV infection.19

Our results show that administration of anti-CD81 MAb s before a mosquito bite challenge of chimeric mice is able to prevent *P. falciparum* sporozoites from infecting the liver of these animals, thereby indicating CD81 as a valuable target for antimalarial therapy.

The humanized mouse model is a useful tool to identify host factors necessary for *P. falciparum* hepatocyte infection and to confirm or refute observations made *in vitro* or *in vivo* using rodent-specific parasite strains. Once the definitive role of one or more entry factors has been established, this model can contribute to the identification and validation of new prophylactic and therapeutic compounds.

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Transparency declarations

None to declare.

References


