Introduction

Plasmodium species have developed multiple strategies to evade and suppress host immunity, which makes treatment and vaccine development very difficult (1). During a blood meal, an infected mosquito injects around 100 sporozoites into the skin, from which the parasites migrate to the bloodstream and travel to the liver (2, 3). After invasion of a hepatocyte, the parasite enters the pre-erythrocytic stage, which lasts 6.5 days (4). The study of Plasmodium falciparum's liver stage is hampered by the low in vitro infection rate of human or primate host cells and by the need for a specialized insectary to rear and infect Anopheles mosquitoes for infection with human or primate host cells and by the need for the production of sporozoites. The development of a mouse model with fully functional human hepatocytes has made it possible to study the liver stage in a preclinical in vivo setting (5–11).

Several candidate malaria vaccines are in development, but most study results have been rather disappointing (12–15). In phase 3 clinical trials, the most advanced malaria vaccine candidate RTS,S/AS01 (RTS,S) in children has demonstrated modest efficacy against clinical and severe malaria. RTS,S targets the pre-erythrocytic phase of the disease and induces high antibody titers against the P. falciparum circumsporozoite protein (CSP) and a moderate CD4+ T cell response. The individual contribution of these adaptive immune responses to protection from infection remains unknown. Here, we found that prophylactic administration of anti-CSP mAbs derived from an RTS,S-vaccinated recipient fully protected mice with humanized livers from i.v.- and mosquito bite–delivered P. falciparum sporozoite challenge. Titers of anti-CSP that conveyed full protection were within the range observed in human RTS,S vaccine recipients. Increasing anti-CSP titers resulted in a dose-dependent reduction of the liver parasite burden. These data indicate that RTS,S-induced antibodies are protective and provide sterilizing immunity against P. falciparum infection when reaching or exceeding a critical plasma concentration.

Conflict of interest: The authors have declared that no conflict of interest exists.

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Vaccine-induced monoclonal antibodies targeting circumsporozoite protein prevent Plasmodium falciparum infection

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17 mice were given varying doses of the anti-CSP mAb Mal1C: 2 mg (n = 11), 200 μg (n = 3), and 20 μg (n = 3). The following day, plasma concentrations of circulating mAbs were measured using a validated and standardized ELISA (25). Immediately thereafter, mice were challenged either via i.v. injection of 150,000 sporozoites (7 PBS, 6 Mal1C) or by exposing each mouse to 20 P. falciparum–infected mosquitoes that were allowed to feed for 20 minutes (6 PBS, 11 Mal1C) (12). 5 days after challenge, mice were euthanized, and their livers were divided into 12 standardized sections. From each of these fragments, 25 μg was used to determine the human hepatocyte content and the P. falciparum liver load using quantitative RT-PCR (qPCR; L. Foquet, unpublished observations, and refs. 30, 31). Regardless of infection route, all PBS-treated mice were infected with P. falciparum, and all mice pretreated with 2 mg Mal1C were protected (Table 1). Anti-CSP plasma concentrations (geometric mean titer [GMT]) measured immediately before challenge were 3,421.5 and 3,133.4 EU/ml in the i.v.- and mosquito-bite–infected groups, respectively. After injection of 200 μg Mal1C, 2 of 3 mice were protected from infection. Antibody concentrations measured in the protected mice before infection were 139.7 and 273.1 EU/ml. The single unprotected mouse had an anti-CSP titer of 230.0 EU/ml and showed a much lower liver parasite burden than sham-treated mice (29.4 P. falciparum/10^6 human hepatocytes; Table 1). Liver parasite burden after treatment with 20 μg mAb in 3 mice showed minimal reduction compared with sham-treated animals. Anti-CSP mAb concentrations in these mice were 31.5, 23.7, and 13.1 EU/ml (Table 1). Next, we tested whether 2 additional mAbs are able to prevent P. falciparum infection when administered in a dose corresponding to serum concentrations achievable by RTS,S vaccination (18, 32). Both Mal2A (HV3-HD3-HJ4:KV3-KJ2) and Mal3B (HV3-HD1-HJ6:KV1-KJ1) were different from Mal1C (HV3-HD3-HJ4:KV2-KJ2), as determined by sequence analysis of V_H:V_L pairs (33). Groups of 3 mice were injected i.p. with 400 μg of Mal1C, Mal2A, or Mal3B and challenged the next day by infected mosquito bites (Table 1). The anti-CSP plasma concentrations (GMT) measured before infection were 668.1, 723.1, and 868.4 EU/ml, respectively. As a control, 1 humanized mouse was injected with PBS and 1 with 400 μg of a control mAb directed against HBsAg, as anti-HBsAg antibodies are also induced by RTS,S. All mice treated with the anti-CSP mAbs were protected against infection, whereas both control mice were infected at day 5 after challenge.

Previous research showed that passive transfer of antibodies directed against the repeat region of P. berghei CSP is capable of arresting P. berghei sporozoite motility within the skin of mice after mosquito challenge (34). Moreover, i.p. administration to human hepatocyte SCID mice of 2.5 mg of an anti-CSP mAb that cross-reacts with P. falciparum and P. berghei (Pf 49 1B2.2) reduced the number of infected human hepatocytes after i.v. injection of 180,000 P. falciparum sporozoites, but sterile protection was not achieved (35). Here, we found that human anti-CSP mAbs derived from an RTS,S vaccinee were able to prevent infection of human liver uPA-SCID mice by P. falciparum when injected prior to parasite challenge, regardless of infection route (i.v. or mosquito bite). The short contact time of antibodies with parasites after non-natural i.v. injection of sporozoites can conceal the protective effect of antibodies with lower binding affinity that may prove effective when the parasites are delivered via mosquito bite.

The anti-CSP concentrations measured immediately before parasite challenge and induced by administration of 400 μg of Mal1C, Mal2A, and Mal3B (Table 1) were in the same range as those previously measured in RTS,S vaccine trials using the same quantification method (25). The lower vaccine efficacy
observed in field trials may be due to a progressive decline in antibody titer during the follow-up period. Indeed, after 12 months, a 95% reduction of anti-CSP titer was observed, and antibody concentrations may drop below the level required to convey sterilizing immunity (36). Our results demonstrated that preventing natural

\textit{P. falciparum} infection of humanized mice could be achieved by passive transfer of mAbs induced by RTS,S vaccination of a malaria-naive volunteer. The sterilizing immunity transferred to the humanized mice provides a proof of principle that anti-CSP antibodies induced by RTS,S are able to prevent \textit{P. falciparum} infection of the liver. These findings further suggest that the protective efficacy of the RTS,S vaccine can possibly be improved by increasing the magnitude and persistence of the CSP-specific antibody response.

Table 1
Prevention of infection by administration of different RTS,S vaccine–induced mAbs

<table>
<thead>
<tr>
<th>Anti-CSP mAb (dose)</th>
<th>Mouse ID</th>
<th>Human albumin (mg/ml)</th>
<th>Liver repopulation (%)</th>
<th>Weight (g)</th>
<th>Preinfection titer (EU/ml)</th>
<th>Liver parasite burden (Pf/10^6 HuHEP)</th>
<th>Positive samples</th>
</tr>
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| i.v. challenge &nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&nbsp;&n
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