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

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Malaria, which is the result of *Plasmodium falciparum* infection, is a global health threat that resulted in 655,000 deaths and 216 million clinical cases in 2010 alone. Recent phase 3 trials with 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.

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 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 (GSK Vaccines; referred to herein as RTS,S), has shown 31% and 50% protective efficacy against clinical malaria in infants (6–12 weeks old) and children (5–17 months old), respectively (16, 17). RTS,S is based on the hepatitis B surface antigen (HBsAg) and the *P. falciparum* circumsporozoite protein (CSP) antigen virus-like particle (VLP) platform (1). In vaccinated humans, RTS,S induces high IgG concentrations to the NANP CSP repeat region and $CD4^+$ T cells that interfere with the ability of the malaria sporozoites to infect hepatocytes (pre-erythrocytic stage) (1, 18). The exact mechanism of protection is still unknown, and in vitro correlates of protection have not yet been defined. Although the titer of anti-CSP IgG is not an established correlate of protection, an association with efficacy has been observed in several trials (15, 19–23), and it is suggested that the protective threshold for anti-CSP IgG concentrations in plasma is >20 μg/ml (24). In addition, an independent and weaker association between CSP-specific $CD4^+$ T cell responses and protection was observed in 2 phase II trials of RTS,S vaccines (21, 23).

Results and Discussion

To evaluate the protective efficacy of anti-CSP IgG in the absence of any confounding (i.e., T cell–mediated) factors, we administered varying doses of 3 human anti-CSP mAbs (designated Mal1C, Mal2A, and Mal3B) into humanized uPA-SCID mice before exposure to *P. falciparum*. The human mAbs recognizing the NANP repeat region of CSP were derived from a subject vaccinated with RTS,S, as described previously (25). uPA-SCID mice were transplanted with cryopreserved primary human hepatocytes, all from the same lot, as described previously (5). The capacity of anti-CSP mAbs to interact with sporozoites and inhibit their motility and cell traversal has previously been examined in vitro using a gliding assay (26) and an inhibition of sporozoite invasion assay (27, 28). The RTS,S-induced anti-CSP mAb Mal1C dose-dependently inhibited gliding motility, with complete inhibition at 1,000 EU/ml (Figure 1, A, C, and D). In the inhibition of sporozoite invasion assay, parasites were added to wells containing HepG2 or Huh7.5 cells. The frequency of parasites invading Huh7.5 and HepG2 cells (27) was comparable (Figure 1, B, E, and F). Since both assays allow for a long interaction time between antibody and sporozoite, one may wonder whether antibody concentrations that inhibit sporozoite functions in vitro are also effective in vivo, when antibodies and sporozoites can only interact during the brief passage from the mosquito bite to the liver (29).

To examine whether human anti-CSP mAbs are capable of preventing in vivo infection with *P. falciparum* sporozoites, 13 humanized uPA-SCID mice were injected i.p. with PBS, and

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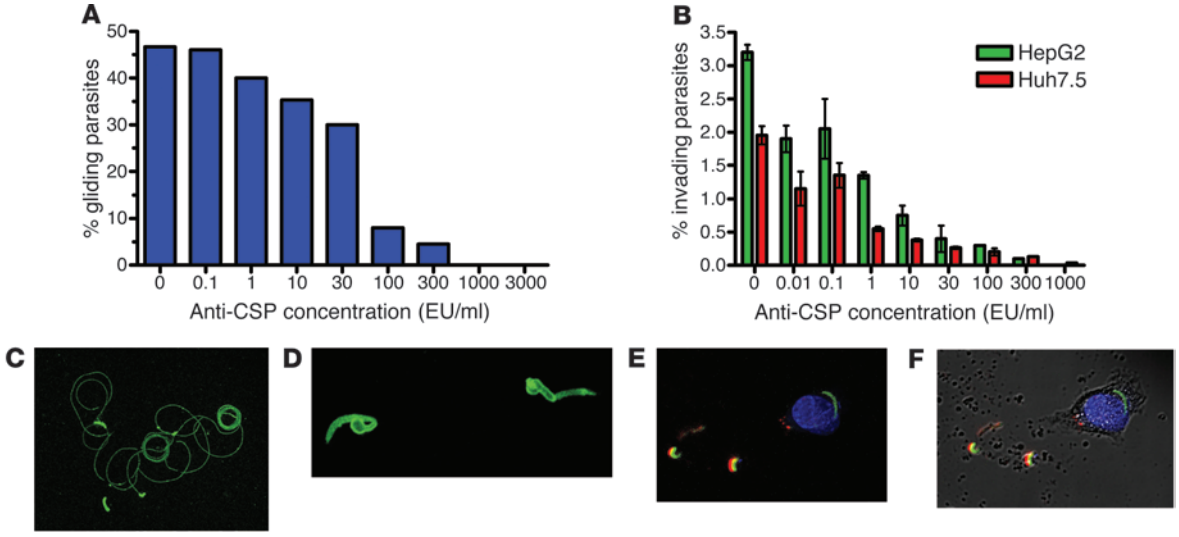

Figure 1
In vitro analysis of functional effects of RTS,S vaccine–induced mAbs on *P. falciparum* sporozoites. (**A** and **B**) A gliding motility assay (**A**) and an inhibition of sporozoite invasion assay (**B**; HepG2 and Huh7.5 cells) were performed after preincubation with different concentrations of Mal1C. Data represent mean ± SD from triplicate wells. (**C** and **D**) Gliding assay. Shown are images of the trails produced by sporozoites in the absence (**C**) and presence (**D**) of anti-CSP mAbs (1,000 EU/ml). (**E** and **F**) Inhibition of sporozoite invasion assay. (**E**) Merged image showing extracellular (red and green) and intracellular (green) parasites. Nuclei were stained with DAPI (blue). (**F**) Additional overlay with a light microscopic image shows the cell membrane. Original magnification, ×63.

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 mg 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

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

Anti-CSP mAb (dose)	Mouse ID	Human albumin (mg/ml)	Liver repopulation (%)	Weight (g)	Preinfection titer (EU/ml)[A]	Liver parasite burden (Pf/10^6 HuHEP)	Positive samples
i.v. challenge[B]							
PBS	B647L	2.4	31.1	12.1	<50	422.8	2 of 12
PBS	K1458RL	3.5	33.2	9.7	<50	251.6	4 of 12
PBS	B627	6.8	33.5	11.8	<50	11,991.8	12 of 12
PBS	B625R	3.1	34.7	18.9	<50	3,261	12 of 12
PBS	B647RL	6	37.9	11.7	<50	3,233.2	12 of 12
PBS	B673L	3.9	38.7	12.1	<50	26.9	2 of 12
PBS	B644L	6.9	59.7	10.4	<50	2,505.3	12 of 12
Mal1C (2 mg)	B698	6.6	51.3	14.4	1,522.9	0	0 of 12
Mal1C (2 mg)	B701L	3.7	40.1	9.6	3,053.1	0	0 of 12
Mal1C (2 mg)	B627R	4.6	32.6	12.3	3,333.9	0	0 of 12
Mal1C (2 mg)	K1447L	2.5	27.7	11.2	3,513.8	0	0 of 12
Mal1C (2 mg)	K1443	3.1	34.9	11.1	3,816.0	0	0 of 12
Mal1C (2 mg)	K1447	4.9	47.6	12.2	4,553.6	0	0 of 12
Mosquito blood meal challenge 1							
PBS	K1468L	4.9	37	16.5	<5	890.7	6 of 12
PBS	K1478	2.8	38.4	14.2	<5	664.6	4 of 12
PBS	K1484L	5.2	55.9	15.1	<5	1,906.4	10 of 12
PBS	K1482L	3.8	33.1	9.5	<50	3,637.6	12 of 12
PBS	K1482	2.9	36.2	10.1	<50	2,909.4	12 of 12
PBS	K1468L	9.4	52.5	13.8	<50	1,966.1	11 of 12
Mal1C (20 μg)	K1477RL	7.2	61.2	11	13.1	543.5	8 of 12
Mal1C (20 μg)	B722	3.2	36.3	13.6	23.7	1,353.2	3 of 12
Mal1C (20 μg)	B673	3.7	38.3	13.4	31.5	150.3	3 of 12
Mal1C (200 μg)	B685	3.9	42.3	16.3	139.7	0	0 of 12
Mal1C (200 μg)	K1479L	3.3	30.9	15.3	230.0	29.4	2 of 12
Mal1C (200 μg)	K1464L	6.6	33.1	13.5	273.1	0	0 of 12
Mal1C (2 mg)	B664RL	6.4	51.3	14.9	2,311.5	0	0 of 12
Mal1C (2 mg)	B710R	3	43.5	14.5	2,813.6	0	0 of 12
Mal1C (2 mg)	B674LL	3.3	36.6	11.1	3,017.2	0	0 of 12
Mal1C (2 mg)	B674L	4.5	33.9	11.5	4,030.2	0	0 of 12
Mal1C (2 mg)	K1475	9.2	62.9	7	5,929.1	0	0 of 12
Mosquito blood meal challenge 2							
PBS	K1622RL	1.9	29.9	11.3	<5	623	7 of 12
Anti-HBsAg (400 μg)	K1651L	7	42.7	12.1	<5[C]	4,938.6	11 of 12
Mal1C (400 μg)	K1670	2.8	34.4	14.5	518.1	0	0 of 12
Mal1C (400 μg)	B894R	2.1	34.5	10.9	734.3	0	0 of 12
Mal1C (400 μg)	B880	4.4	43.3	8.5	784.0	0	0 of 12
Mal2A (400 μg)	K1617L	5.9	47.2	12.6	400.2	0	0 of 12
Mal2A (400 μg)	B894	2.4	35.7	11.1	1,099.9	0	0 of 12
Mal2A (400 μg)	B880R	4.2	44.2	9.2	859.1	0	0 of 12
Mal3B (400 μg)	K1617RL	4.8	36.4	13.1	567.0	0	0 of 12
Mal3B (400 μg)	B864L	2.8	29.2	11.8	926.0	0	0 of 12
Mal3B (400 μg)	K1552	2.1	38.7	10.2	1,247.3	0	0 of 12

Pf/10^6 HuHEP, *P. falciparum* per 10^6 human hepatocytes. [A]Depending on dilution, detection limit was 5 or 50 EU/ml. [B]150,000 sporozoites. [C]Titer was 1.65×10^6 mIU/ml.

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 *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 *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.

Methods

Further information can be found in Supplemental Methods and Supplemental Table 1, available online with this article; doi:10.1172/JCI70349DS1.

Generation of humanized mice. Humanized uPA-SCID mice were generated as described previously (5).

Generation of human anti-CSP mAbs. The cell donor of the PBMCs used to generate the human anti-CSP mAb was selected from a clinical trial (MAL-080) evaluating the RTS,S vaccine at the Center for Vaccinology, Ghent University and Ghent University Hospital. Human B lymphocytes were immortalized as described previously (37). The mAb concentration was determined by measuring UV absorbance at 280 nm (1 mg/ml = 1.4 absorbance units) and by anti-CSP ELISA (25).

mAb sequencing. Ig variable genes from 3 anti-CSP–producing hybridomas were sequenced at the Centre for Medical Genetics of Ghent University, and obtained sequences were analyzed using IMGT/V-Quest (http://www.imgt.org/) to assign the variable gene family (33).

Anti-CSP ELISA. Antibodies specific for the CSP tandem repeat epitope were assessed by a validated, standard ELISA (25). At days 0 and 5, mice were bled, and plasma was stored at –80°C until analysis.

In vitro assays of anti-CSP antibody. Gliding assays and inhibition of sporozoite invasion assays were performed as described previously (38).

In vivo parasite challenge and prophylactic treatment experiments. 1 day prior to parasite challenge, chimeric uPA-SCID mice were injected i.p. with PBS, anti-HBsAg mAb, or mAbs specifically targeting the *P. falciparum* CSP. The following day, all animals were challenged with sporozoites, either via bites by infected mosquitos or via injection of parasites into the retro-orbital venous sinus. *Anopheles stephensi* mosquitoes were reared at Radboud University Medical Centre and infected according to previously described standard procedures (39).

Isolation and detection of P. falciparum DNA and human hepatocyte DNA by qPCR. 5 days after infection, mice were euthanized by cervical dislocation, and their livers were stored at 4°C until analysis. *P. falciparum* DNA levels were quantified using a highly sensitive qPCR assay (30). To assess the degree of repopulation with human hepatocytes of the chimeric livers, and to normalize the *P. falciparum* copy numbers, we used qPCR as previously described (31).

Statistics. Data are shown as mean ± SD. Analysis were performed using GraphPad Prism.

Study approval. All procedures were approved by the Animal Ethics Committee of the Faculty of Medicine and Health Sciences of Ghent University.

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