Supplemental data

Expanded Methods

Generation of humanized mice

Humanized uPA-SCID mice were generated as described (1). Briefly, within two weeks after birth cryopreserved primary human hepatocytes (± 10^6 cells/mouse, all from the same donor and purchased from Becton-Dickinson) were injected in the spleens of $uPA^{+/+}$-SCID mice (2). Five weeks later mouse plasma was analyzed for the presence of human albumin using Human Albumin ELISA Quantitation kit (Bethyl Laboratories Inc., Montgomery, TX). Animals with a human albumin level >2 mg/mL were considered successfully engrafted and used for infection studies.

Generation of human monoclonal antibodies directed against the CS protein

The cell donor of the PBMC used to generate the human monoclonal antibody directed against CSP was selected from a clinical trial (MAL-080) evaluating the RTS,S vaccine at the Center for Vaccinology (Ghent University and Ghent University Hospital, Belgium). Human B lymphocytes were fused with the K6H5/B5 heteromyeloma line as described previously (3) and fusion products were selected for anti-CSP antibody production using an in-house anti-CSP ELISA (see below). Three anti-CSP producing hybridomas (Mal1C, Mal2A, Mal3B) were grown in Integra CELLine CL1000 (Integra Biosciences, Chur, Switzerland). Monoclonal antibodies were purified from the culture supernatants by fast protein liquid chromatography on 1 mL HiTrap protein G HP columns (GE Healthcare), followed by desalting with Amicon Ultra-15 Centrifugal filter (Millipore). The mAb concentration in the resulting solution was determined by measuring UV absorbance at 280 nm (1 mg/ mL = 1.4 absorbance units), and by the anti-CSP ELISA (4).

Monoclonal antibody sequencing

Total RNA was isolated from three anti-CSP producing hybridomas using RNeasy mini kit

(Qiagen, Venlo, Netherlands). Random priming of obtained RNA was performed using Superscript III (Invitrogen, Karsruhe, Germany) according to standard procedures. Amplification of the variable regions of human IgG Heavy (V_H) and Light (V_L) chain was done via standard PCR using previously described human Ig primerset based on family assigned genes (5) and Phusion polymerase (New England Biolabs , Frankfurt, Germany). All primers used were obtained from Biolegio (Nijmegen, Netherlands). Amplified fragments of about 650 to 700bp were gel extracted (Qiaquick, Qiagen, Venlo, Netherlands) and ligated into PCR-Blunt® vector (Invitrogen, Karsruhe, Germany) for sequencing. Between 3-6 clones were sequence analysed for each insert by the Centre for Medical Genetics (Ghent University, Belgium). Obtained sequences were analysed using IMGT/V-Quest (http://www.imgt.org/) to assign the variable gene family (6).

Anti-CSP ELISA

Antibodies specific for the circumsporozoite protein tandem repeat epitope were assessed by a validated, standard ELISA (4). Briefly, the recombinant antigen R32LR that contains the sequence [NVDP(NANP)15]2LR was coated onto a 96-well polystyrene plate. After blocking the plate with blocking buffer (PBS (pH 7.4) containing 0.1% Tween-20 (v/v) and 0.5% skimmed milk (Becton Dickinson, ref 232100), serial dilutions of serum samples were added to the plate. The plates were washed and polyclonal rabbit anti-human IgG/HRP was added. After a final washing step and a color reaction with 3, 3',5,5' tetramethylbenzidine, the plates were read in an ELISA reader. The titers were calculated from a standard curve with the software SoftmaxPro (using a four parameters equation) and expressed as EU/ml. The cut-off for the anti-CSP ELISA was 0.5 EU/ml. At day 0 and day 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 (7). Briefly, 20,000 sporozoites harvested from *P. falciparum* infected *Anopheles stephensi* mosquito salivary glands were pre-incubated on ice during 30 minutes with a predefined concentration of control antibody or [RTS,S]-induced monoclonal anti-CSP antibody Mal1C. During the gliding assay, the sporozoites were directly transferred to 8-chamber slides coated with 25 µg/mL anti-CSP antibody and incubated at 37°C for 2 hours. After fixation with 4% paraformaldehyde, sporozoites and gliding circles were stained with a FITC-conjugated anti-CSP antibody. Analysis was performed using a confocal Leica TCS SPE microscope.

For the inhibition of sporozoite invasion assay, 70,000 HepG2 or Huh7.5 cells were seeded in 96-well plates 24 hours before the experiment. After 4 hours of incubation at 37°C with 20,000 parasites, the cultures were fixed with 10% paraformaldehyde and extracellular sporozoites were first stained with anti-CSP antibodies followed by secondary Alexa 647 conjugated antibodies (red). Then, cells were permeabilised and intracellular sporozoites were stained with anti-CSP antibodies and secondary FITC conjugated antibodies (green). Cell nuclei were stained with DAPI (blue). Analysis was performed on a BD Pathway 435 benchtop microscope.

In vivo parasite challenge and prophylactic treatment experiments

One day prior to challenge with sporozoites, chimeric uPA-SCID mice were injected intraperitoneal with 2,000; 400; 200 or 20 µg of mAbs specifically targeting the *P. falciparum* CSP protein. Control animals were injected with an equal volume of PBS or 400 µg anti-HBs mAb. The next day, blood samples were taken for anti-CSP quantification. Subsequently, all animals were challenged with sporozoites, either by mosquito bite or direct injection into the retro-orbital venous sinus. To test whether passively immunized mice were protected against infection via the natural transmission route, control and treated mice were exposed for 20 minutes to bites of 20 *P. falciparum*-infected mosquitoes (*Anopheles stephensi*) that contained an average of 70.000

salivary gland sporozoites each. *Anopheles stephensi* mosquitoes were reared at Radboud University Medical Centre (Nijmegen, The Netherlands) and infected by allowing them to feed on cultured gametocytes of *P. falciparum* parasites (NF54) according to standard procedures as described previously (8). Successful blood feeding (mean: 17 mosquitoes, range: 15-20) and sporozoite presence (100 %) was confirmed by mosquito dissection after the challenge experiment. To reach a higher and defined number of injected parasites in the first experiment, sporozoites were isolated by manual dissection from the salivary glands of infected mosquitoes that were killed by ethanol spraying (9). Mice received a retro-orbital intravenous injection of 150,000 sporozoites in a volume of 100 µl RPMI 1640 (GIBCO).

Isolation and detection of *P. falciparum* DNA and human hepatocyte DNA by qPCR

Five days after infection, mice were euthanized by cervical dislocation and their livers were carefully removed, reproducibly divided into 12 sections that were stored in RNALater (Sigma) at 4°C until analysis. Of each section, 25 mg tissue was weighed and DNA was extracted into 100µL elution buffer with the High Pure PCR Template Preparation Kit (Roche). *P. falciparum* DNA levels were quantified using a highly sensitive qPCR assay (10). Briefly, 5µL DNA extract was added to 20µL master mix containing *P. falciparum* 18S RNA gene-specific primers and a probe labeled with 6-carboxy-fluorescein (FAM) as a reporter and 6-carboxy-tetramethylrhodamine (TAMRA) as a quencher. *P. falciparum* standard curves were prepared by DNA extraction from titrated samples of ring-infected cells (range 5–12.000 parasites per 100µL extract).

To assess the degree of repopulation with human hepatocytes of the chimeric livers, and to normalize the *P. falciparum* copy numbers, we employed a qPCR as described by Alcoser, *et al* (11). Summarized, 1µL containing 50 ng of DNA was added to 19µL master mix containing common primers that amplify a 215 bp region located in the human and mouse prostaglandin E receptor 2 (PTGER2) genes. The amplified region contains a non-homologous sequence which is targeted by two species-specific Taqman probes, each conjugated with a unique fluorescent tag

(FAM and Cy5), which makes it possible to quantify the copy number and differentiate between mouse and human origin in duplex. Standard curves were prepared by DNA extraction from a titration of a defined amount of human PBMC and mouse spleen cells. Percentage calculation was verified by making various ratios of mouse and human DNA extracts.

Statistics

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

Study approval

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

Table 1: Sequences of V_H:V_L pairs of three monoclonal antibodies derived from a RTS,S vaccine recipient.

Monoclonal Antibody ID	Gene Family Assignment*
Mal1C	HV3-HD3-HJ4 : KV2-KJ2
Mal2A	HV3-HD3-HJ4 : KV3-KJ2
Mal3B	HV3-HD1-HJ6 : KV1-KJ1

*each heavy and light chain was distinct

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