Published in final edited form as:

Int J Parasitol. 2013 October ; 43(11): 869–874. doi:10.1016/j.ijpara.2013.06.004.

Immunization against a serine protease inhibitor reduces intensity of *Plasmodium berghei* infection in mosquitoes

Andrew R. Williams^{a,*}, Sara E. Zakutansky^a, Kazutoyo Miura^b, Matthew J. D. Dicks^a, Thomas S. Churcher^c, Kerry E. Jewell^a, Aisling M. Vaughan^a, Alison V. Turner^a, Melissa C. Kapulu^a, Kristin Michel^d, Carole A. Long^b, Robert E. Sinden^{a,e}, Adrian V. S. Hill^a, Simon J. Draper^a, and Sumi Biswas^a

^aThe Jenner Institute, University of Oxford, Roosevelt Drive, Oxford, OX3 7DQ, UK

^bLaboratory of Malaria and Vector Research, NIAID, NIH, Rockville, MD 20852, USA

^cDepartment of Infectious Disease Epidemiology, Imperial College London, London, UK

^dKansas State University, Division of Biology, Manhattan, KS 66506, USA

^eDivision of Cell and Molecular Biology, Imperial College London, London, UK

Abstract

The mosquito innate immune response is able to clear the majority of *Plasmodium* parasites. This immune clearance is controlled by a number of regulatory molecules including serine protease inhibitors (serpins). To determine whether such molecules could represent a novel target for a malaria transmission-blocking vaccine, we vaccinated mice with *Anopheles gambiae* serpin-2 (AgSRPN2). Antibodies against AgSRPN2 significantly reduced the infection of a heterologous *Anopheles* species (*Anopheles stephensi*) by *Plasmodium berghei*, however this effect was not observed with *Plasmodium falciparum*. Therefore, this approach of targeting regulatory molecules of the mosquito immune system may represent a novel approach to transmission-blocking malaria vaccines.

Keywords

Malaria; Vaccine; Mosquito; Antibodies; Serpins

Malaria, caused by protozoan parasites of the genus *Plasmodium*, is one of the world's most devastating infectious diseases. It is estimated that more than 500 million people per year develop malaria, with close to one million deaths (Murray et al., 2012). Eradication of malaria has been proposed, but for this goal to be achieved a highly effective vaccine will be necessary. *Plasmodium* has a complex lifecycle that involves asexual replication in both the liver and red blood cells of the vertebrate host and sexual reproduction within the midgut of

© 2013 Australian Society for Parasitology. Published by Elsevier Ltd. All rights reserved

*Corresponding author. Current address: Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Groennegaardsvej 15, DK-1870 Frederiksberg C, Denmark. Tel.: +45 353 32797 arw@sund.ku.dk.

Conflict of Interest statement: ARW, AVSH and SJD are named inventors on patent applications covering malaria vectored vaccines and/or immunization regimes.

Supplementary data associated with this article

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the *Anopheles* mosquito vector. Vaccines can target the parasite at any one of three different stages; the pre-erythrocytic stage (either the sporozoite or the infected hepatocyte), the erythrocytic stage or within the mosquito. Vaccines that target the parasite at the mosquito stage prevent transmission of the parasite through the vector – hence, they are known as transmission-blocking vaccines (TBVs). TBVs aim to induce herd immunity amongst a community and their development is likely to be essential for the eradication of malaria (The malERA Consultative Group on Vaccines, 2011). Several TBV candidate antigens have been studied, with the majority consisting of proteins on the surface of gametocytes, gametes or the ookinete, and antibodies against these can substantially reduce transmission in pre-clinical models (Sinden, 2010; The malERA Consultative Group on Vaccines, 2011).

In addition to targeting antigens on the parasite, antigens located in the mosquito (which are essential for parasite development) have also shown potential as TBV candidates. For example, it has been shown that immunizing mice with either an *Anopheles* aminopeptidase (APN1) (Dinglasan et al., 2007), or carboxypeptidase (CPBAG1) (Lavazec et al., 2007), can raise antibodies that block the transmission of *Plasmodium* parasites, suggesting an important role of specific mosquito molecules in parasite invasion of the mosquito midgut. In recent years, much attention has also been focused on understanding the mechanisms behind mosquito innate immunity. After taking a blood meal, mosquitoes mount a potent, non-specific innate immune response that is thought to protect against establishment of bacteria in the midgut as a result of blood-feeding (Dong et al., 2009). This innate immunity can also act against *Plasmodium* parasites and in fact the mosquito immune response normally clears the vast majority of the invading parasites (Alavi et al., 2003). It is therefore possible that this natural resistance may be exploited to prevent the transmission of malaria. Innate parasite rejection is mediated by lysis and melanin neutralization (Blandin et al., 2004). It is apparent that this process is controlled by a number of regulatory molecules that prevent the immune response from over-activation. For example, serpins, a group of serine protease inhibitors present in all eukaryotes, negatively regulate insect immune responses to bacteria and protozoan parasites (Ligoxygakis et al., 2002; Michel et al., 2005). The importance of serpins in controlling the mosquito innate immune response has been demonstrated by RNA interference (RNAi) silencing of the serpin-2 (*SRPN2*) gene in *Anopheles gambiae*, leading to massive formation of pseudotumors and mosquito death (Michel et al., 2005; An et al., 2011). Strikingly, knockdown of *SRPN2* also dramatically reduced the numbers of oocysts during infection with the rodent malaria parasite *Plasmodium berghei* (Michel et al., 2005). Consequently, some groups have proposed the idea of genetically modifying mosquitoes to either over-express genes involved in parasite killing or under-express genes involved in regulation of these highly potent immune mechanisms (Dong et al., 2011), thus rendering them refractory to *Plasmodium* infection and unable to transmit the parasite to humans.

Here we explored whether molecules that regulate the innate immune response within the mosquito could also be candidate antigens for a malaria TBV. We hypothesised that antibodies against these molecules would inhibit their function, result in increased activation of the mosquito innate immune response and reduce transmission of the parasite. We report that immunization of mice with *A. gambiae* *SRPN2* (AgSRPN2) raises antibodies that significantly reduce the intensity of *P. berghei* infection in *Anopheles stephensi*, suggesting that this approach warrants further investigation as a novel strategy for the development of malaria TBVs.

Recombinant adenoviral and poxviral vectors expressing the full-length AgSRPN2 protein, excluding the signal peptide (amino acids, aa 1–21) were generated. The AgSRPN2 gene (AGAP006911-PA/XP_308845), aa 22–409 was codon optimized for expression in humans and synthesized by GeneArt GmbH. A BLAST search indicated that AgSRPN2 had a

maximum of 33% identity with murine proteins, the closest match being a Serpin B11 (**AAH10313.1**), with other serpins having around 25–26% sequence identity. This gene was cloned into ChAd63 and MVA shuttle vectors, downstream of the human tissue plasminogen leader sequence in order to aid secretion of the antigen from virally infected / immunized cells, and the recombinant viruses were generated as previously described for other antigen inserts (Douglas et al., 2011; Goodman et al., 2011). Mice were then immunized in a heterologous prime-boost regime which has previously been shown to induce antibodies in both pre-clinical studies and in Phase I/IIa clinical trials of blood-stage malaria vaccine candidates (Draper et al., 2008; Goodman et al., 2011; Sheehy et al., 2012). Six week old female BALB/c mice were obtained from Harlan, UK. Mice were first primed with i.m. injection of 1×10^8 infectious units (iu) of ChAd63 and then 8 weeks later were boosted i.m. with 1×10^7 plaque forming units (pfu) of MVA. Control mice were immunized with vectors expressing an irrelevant antigen (GFP).

Immunogenicity of the vectors was confirmed by ELISA using recombinant AgSRPN2 protein as antigen. Nunc Maxisorp plates (Fisher Scientific, UK) were coated at 100 ng per well with recombinant active AgSRPN2 (produced as described by An et al. (2011)) and left overnight. Plates were washed six times the next day with PBS containing 0.05% Tween 20 (PBS/T) and blocked with 10% skimmed milk in PBS/T for 1 h. Sera were added and incubated for 2 h. Alkaline phosphatase-conjugated goat anti-mouse IgG at 1:5000 dilution was used for detection. Plates were washed and bound antibodies were detected by adding p-nitrophenylphosphate substrate (pNPP, Sigma, UK) diluted in diethanolamine buffer (Fisher Scientific, UK). OD 405 nm (OD₄₀₅) was read using an ELx800 microplate reader (BioTek, UK). End-point titers were taken as the x-axis intercept of the dilution curve at an absorbance value three S.D.s. greater than the OD₄₀₅ for naïve mouse serum.

Anti-AgSRPN2 antibody titres were below the assay detection limit in mice immunized with GFP (data not shown). In contrast, anti-AgSRPN2 IgG antibodies were induced by the priming AgSRPN2 immunization, with titers significantly higher at day 55 than at day 14 ($P < 0.05$ by repeated-measures ANOVA), and these were boosted further by the MVA immunization (Fig. 1). We thus confirmed that vectors expressing a component of the mosquito immune system are immunogenic in mammals, using a vaccine delivery platform that is safe and induces antibodies in humans (Sheehy et al., 2012).

Next, the functional transmission-blocking activity of the vaccine-induced antibodies was tested. First, we used an ex vivo direct membrane feeding assay (DMFA) using the rodent malaria parasite *P. berghei*. Two weeks after the final immunization, mice were bled by cardiac puncture under terminal anaesthesia to harvest serum. In parallel, the *P. berghei* ANKA strain (clone 2.34) was maintained by serial passage in TO mice. For membrane feeding assays, hyper-reticulocytosis was induced in mice by injection of 6 mg/mL of phenylhydrazine 3 days prior to infection with 10^7 parasitized red blood cells (pRBCs). Three days after infection, exflagellation of male gametocytes was checked as described previously (Blagborough and Sinden, 2009). Blood containing infectious *P. berghei* gametocytes was then collected by cardiac puncture, split into two equal aliquots and mixed with pooled serum from mice immunized with either AgSRPN2 or GFP (each $n = 8$) at a dilution of 1:5 serum: blood and fed to *A. stephensi* mosquitoes through plastic membrane feeders. Unfed mosquitoes were removed the next day and the infected mosquitoes were then maintained at 19°C and 70% relative humidity. Midguts were dissected 11 days after infection and stained with 0.05% mercurochrome for enumeration of oocysts. DMFAs were performed on three separate occasions. Parasite burdens were significantly reduced in mosquitoes that were fed with blood containing anti-AgSRPN2 antibodies compared with serum from GFP-immunized controls, with an average reduction in oocyst intensity (i.e. the number of oocysts per mosquito) of 54% (95% confidence interval, (CI) 34 – 67%; $P <$

0.0002 using a generalized-linear mixed model, GLMM (Churcher et al., 2012); Fig. 2). There was only a modest reduction of 10% (CI = 0.3 – 30%) in oocyst prevalence (the number of infected mosquitoes, i.e. those with at least one oocyst), which was not significant ($P = 0.055$ using a GLMM with binomial error structure).

We next sought to establish whether *P. berghei* transmission would be inhibited when mosquitoes fed directly on live mice, thus replicating the natural route of infection. Five mice were immunized with AgSRPN2 (ChAd63-MVA) and four mice with control vectors as described above. Two weeks after the final immunization, the same mice were infected with blood-stage *P. berghei*. The presence of infective gametocytes in blood was confirmed and a different group of mosquitoes were then allowed to feed directly on each anesthetized mouse. All five groups of mosquitoes that fed on the mice immunized with AgSRPN2 had lower numbers of oocysts compared with mosquitoes that fed on the control mice (Fig. 3A). Altogether, the AgSRPN2 mice had an oocyst intensity 42% (CI = 23 – 52%) lower than control mice ($P = 0.0002$, estimated by allowing the infectivity of different mice to vary at random in a non-pairwise GLMM). There was no significant effect on the prevalence of infection ($P = 0.42$).

Having confirmed that vaccination with AgSRPN2 induced antibodies with significant anti-parasitic activity against *P. berghei*, these antibodies were also tested in two independent standard membrane feeding assays (SMFA) with cultured *Plasmodium falciparum* (NF54 strain) and *A. stephensi* mosquitoes. The SMFA was performed essentially as described (Cheru et al., 2010). Briefly, IgG was purified by protein G affinity chromatography from the same pooled mouse serum as was used for the *P. berghei* DMFA, and in vitro cultured *P. falciparum* gametocytes (NF54 strain) mixed with test IgG (0.75 mg/mL) were fed to *A. stephensi* mosquitoes. However, in contrast to the results with *P. berghei*, here no overall transmission-blocking activity was observed (Fig. 3C), either in terms of reducing oocyst intensity ($P = 0.2$ by GLMM) or prevalence ($P = 1$ by GLMM).

Here we have described proof-of-concept for a novel approach to malaria TBV development that may augment existing strategies to target the parasite in the mosquito vector. While others have highlighted the potential of increasing the resistance of mosquitoes to *Plasmodium* infection through targeted gene-knockout (for example Rodrigues et al., 2012), we believe this is the first report of accomplishing this through vaccination of the vertebrate host against a regulatory component of the innate immune response. Importantly, this was achieved through use of a human-compatible vaccine delivery platform that has previously been shown to be safe and immunogenic in delivering a variety of liver- and blood-stage malaria antigens, as well as transmission-blocking antigens such as Pfs25 (Goodman et al., 2011; Sheehy et al., 2012). Moreover, whilst the vaccine was based on the *A. gambiae* SRPN2 sequence, significant efficacy was observed in *A. stephensi*, raising the possibility that antibodies raised by this immunization strategy may be reactive against a variety of mosquito species. Alignment of the AgSRP2 amino acid sequence with the corresponding protein from *A. stephensi* (AsSRP2) revealed a very high level of homology; 92% sequence identity and 97% sequence similarity between the two proteins (Supplementary Fig. S1; Supplementary Table S1; see Supplementary Data S1 for details of the alignment methods). In addition, both the AgSRP2 and AsSRP2 proteins had a high level of amino acid sequence homology with the SRP2 protein in a third *Anopheles* sp., *Anopheles darlingi* (Supplementary Fig. S1; Supplementary Table S1). This suggests a very high level of conservation amongst different *Anopheles* spp. However, further optimization of this approach will be required to identify other mosquito immune regulators as vaccine targets that can induce antibodies capable of blocking the transmission of *P. falciparum*. It will be interesting to ascertain the level of polymorphism in other immune-regulatory molecules in

different mosquito species, in order to determine whether they may also represent conserved targets for a vaccine.

The magnitude of the transmission-blocking activity observed with *P. berghei* (median reduction of intensities by 54% in DMFA and 42% in in vivo feeding experiments) is comparable with that achieved by other TBV candidate antigens within the mosquito, although it must be noted that quite different analysis methodologies have been applied in previously reported studies. Dinglasan et al. (2007) reported a 75% reduction in oocyst intensity in mosquitoes fed directly on *P. berghei*-infected mice passively immunized with *A. gambiae* (Ag)APN1, whilst we have previously shown a 67% reduction in oocyst intensity in mosquitoes fed on mice infected with transgenic *P. berghei* expressing Pfs25, following Pfs25 immunization using a similar viral-vectored regime to that described here (Goodman et al., 2011). Given that Pfs25 is one of the leading parasite TBV candidate antigens (Sinden, 2010), our results suggest that targeting the mosquito innate immune system through vaccination has the potential to achieve levels of anti-parasitic activity comparable with current leading strategies. Although we did not observe a corresponding reduction in prevalence in this study, this could be attributed to the very high oocyst intensity in the control group. The relationship between intensity and prevalence of *Plasmodium* infection in laboratory experiments has been analysed by Churcher et al. (2012), which showed that at high infection intensities (means of 50 – 100 oocysts), extremely high reductions in intensity are necessary to observe even a modest decrease in infection prevalence. Extrapolating from this, at the oocyst intensities observed in the control mosquitoes (means of 72 and 120 for the DMFA and in vivo feeding experiments, respectively), we would need to achieve a >95% reduction in infection intensity in order to have a reduction in prevalence of ~20%. Given oocyst intensity is normally much lower in the field than in laboratory experiments (Rosenberg, 2008), it is likely that the reduction in intensity observed with this new approach could translate into a corresponding reduction in prevalence under field conditions, and thus impart meaningful transmission-blocking activity. Moreover, since transmission-blocking activity is normally closely correlated with antibody titers induced by vaccination (Miura et al., 2007), it is possible that increasing antibody responses by boosting with a recombinant protein-in-adjuvant vaccine (Draper et al., 2010) might enhance the observed response. It will, however, need to be demonstrated that this concept is also effective with *P. falciparum* if it is to be considered a viable alternative and/or complementary strategy to existing TBV approaches.

The mechanism of increased parasite killing was not investigated here, but as there was no effect of AgSRPN2 immunization on blood-stage parasitemia or gametocytemia (data not shown), our results suggest that the anti-parasitic effect is mediated within the mosquito by antibodies ingested in the blood meal. Previous work has shown that knockdown of AgSRPN2 by RNAi leads to increased melanization of invading *P. berghei* ookinetes in the midgut basal lamina (Michel et al., 2005), and it is possible that similar mechanisms may be involved in the reduced oocyst formation we observed in the presence of anti-AgSRPN2 antibodies. AgSRPN2 is present in the mosquito hemolymph where it prevents melanization in the mosquito by inhibiting pro-phenoloxidase (PPO) activation, a rate-limiting enzyme for melanin production (Michel et al., 2006; An et al., 2011). It is possible that anti-AgSRPN2 antibodies diffuse from the blood meal across the midgut epithelium, where they come into contact with and neutralize SRPN2, resulting in increased activation of the PPO system and consequent melanization of parasites. Further experiments are necessary to elucidate these mechanisms. However, in further agreement with our findings showing a lack of transmission blocking activity by anti-AgSRPN2 antibodies against *P. falciparum*, it has been shown that knockdown of *AgSRPN2* does not affect development of *P. falciparum* in *Anopheles* spp. (Michel et al., 2006), consistent with previous studies suggesting there may be differential mechanisms of mosquito immunity against rodent and human malaria

parasites (Dong et al., 2006). Therefore, whilst we have clearly shown the potential to reduce the intensity of *Plasmodium* parasite oocysts within the mosquito by this vaccination approach, further work will be necessary to identify candidate antigens that induce antibodies specifically affecting *P. falciparum* development. The availability of the *A. gambiae* genome provides a wealth of information for selection of possible target antigens. For example, it has been shown that knockdown of a peroxidase/dual oxidase system in *A. gambiae* reduces development of both *P. berghei* and *P. falciparum* (Kumar et al., 2010), and such proteins may be more suitable as vaccine targets using our approach.

In conclusion, we have to our knowledge provided the first proof-of-concept that vaccination against regulatory components of the mosquito innate immune response may reduce the intensity of *Plasmodium* infection in mosquitoes. Further work should focus on testing whether some of the numerous other molecules involved in this immune response may provide more effective TBV targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to the Jenner Institute Vector Core Facility, UK for their assistance, and to Alfredo Nicosia (Okairòs, Italy) for provision of the ChAd63 vector backbone. This work was supported by a grant from the Gates Foundation Grand Challenges in Global Health (USA) awarded to SB. SJD holds a UK MRC Career Development Fellowship (Grant number G1000527). AVSH and SJD are Jenner Investigators.

References

- Alavi Y, Arai M, Mendoza J, Tufet-Bayona M, Sinha R, Fowler K, Billker O, Franke-Fayard B, Janse CJ, Waters A, Sinden RE. The dynamics of interactions between *Plasmodium* and the mosquito: a study of the infectivity of *Plasmodium berghei* and *Plasmodium gallinaceum*, and their transmission by *Anopheles stephensi*, *Anopheles gambiae* and *Aedes aegypti*. *Int. J. Parasitol.* 2003; 33:933–943. [PubMed: 12906877]
- An C, Budd A, Kanost M, Michel K. Characterization of a regulatory unit that controls melanization and affects longevity of mosquitoes. *Cell. Molec. Life Sci.* 2011; 68:1929–1939. [PubMed: 20953892]
- Blagborough AM, Sinden RE. *Plasmodium berghei* HAP2 induces strong malaria transmission-blocking immunity in vivo and in vitro. *Vaccine.* 2009; 27:5187–5194. [PubMed: 19596419]
- Blandin S, Shiao S-H, Moita LF, Janse CJ, Waters AP, Kafatos FC, Levashina EA. Complement-Like Protein TEPI Is a Determinant of Vectorial Capacity in the Malaria Vector *Anopheles gambiae*. *Cell.* 2004; 116:661–670. [PubMed: 15006349]
- Cheru L, Wu Y, Diouf A, Moretz SE, Muratova OV, Song G, Fay MP, Miller LH, Long CA, Miura K. The IC50 of anti-Pfs25 antibody in membrane-feeding assay varies among species. *Vaccine.* 2010; 28:4423–4429. [PubMed: 20434549]
- Churcher TS, Blagborough AM, Delves M, Ramakrishnan C, Kapulu MC, Williams AR, Biswas S, Da DF, Cohuet A, Sinden RE. Measuring the blockade of malaria transmission – An analysis of the Standard Membrane Feeding Assay. *Int. J. Parasitol.* 2012; 42:1037–1044. [PubMed: 23023048]
- Dinglasan RR, Kalume DE, Kanzok SM, Ghosh AK, Muratova O, Pandey A, Jacobs-Lorena M. Disruption of *Plasmodium falciparum* development by antibodies against a conserved mosquito midgut antigen. *PNAS.* 2007; 104:13461–13466. [PubMed: 17673553]
- Dong Y, Aguilar R, Xi Z, Warr E, Mongin E, Dimopoulos G. *Anopheles gambiae* Immune Responses to Human and Rodent *Plasmodium* Parasite Species. *PLoS Pathog.* 2006; 2:e52. [PubMed: 16789837]
- Dong Y, Das S, Cirimotich C, Souza-Neto JA, McLean KJ, Dimopoulos G. Engineered *Anopheles* Immunity to *Plasmodium* Infection. *PLoS Pathog.* 2011; 7:e1002458. [PubMed: 22216006]

- Dong Y, Manfredini F, Dimopoulos G. Implication of the Mosquito Midgut Microbiota in the Defense against Malaria Parasites. *PLoS Pathog.* 2009; 5:e1000423. [PubMed: 19424427]
- Douglas AD, Williams AR, Illingworth JJ, Kamuyu G, Biswas S, Goodman AL, Wyllie DH, Crosnier C, Miura K, Wright GJ, Long CA, Osier FH, Marsh K, Turner AV, Hill AV, Draper SJ. The blood-stage malaria antigen PfRH5 is susceptible to vaccine-inducible cross-strain neutralizing antibody. *Nat. Commun.* 2011; 2:601. [PubMed: 22186897]
- Draper SJ, Biswas S, Spencer AJ, Remarque EJ, Capone S, Naddeo M, Dicks MDJ, Faber BW, de Cassan SC, Folgori A, Nicosia A, Gilbert SC, Hill AVS. Enhancing Blood-Stage Malaria Subunit Vaccine Immunogenicity in Rhesus Macaques by Combining Adenovirus, Poxvirus, and Protein-in-Adjuvant Vaccines. *J. Immunol.* 2010; 185:7583–7595. [PubMed: 21098232]
- Draper SJ, Moore AC, Goodman AL, Long CA, Holder AA, Gilbert SC, Hill F, Hill AV. Effective induction of high-titer antibodies by viral vector vaccines. *Nat. Med.* 2008; 14:819–821. [PubMed: 18660818]
- Goodman AL, Blagborough AM, Biswas S, Wu Y, Hill AV, Sinden RE, Draper SJ. A Viral Vectored Prime-Boost Immunization Regime Targeting the Malaria Pfs25 Antigen Induces Transmission-Blocking Activity. *PLoS ONE.* 2011; 6:e29428. [PubMed: 22216279]
- Kumar S, Molina-Cruz A, Gupta L, Rodrigues J, Barillas-Mury C. A Peroxidase/Dual Oxidase System Modulates Midgut Epithelial Immunity in *Anopheles gambiae*. *Science.* 2010; 327:1644–1648. [PubMed: 20223948]
- Lavazec C, Boudin C, Lacroix R, Bonnet S, Diop A, Thiberge S, Boisson B, Tahar R, Bourgoignie C. Carboxypeptidases B of *Anopheles gambiae* as Targets for a *Plasmodium falciparum* Transmission-Blocking Vaccine. *Infect. Immun.* 2007; 75:1635–1642. [PubMed: 17283100]
- Ligoxygakis P, Pelte N, Ji C, Leclerc V, Duvic B, Belvin M, Jiang H, Hoffmann JA, Reichhart J-M. A serpin mutant links Toll activation to melanization in the host defence of *Drosophila*. *EMBO J.* 2002; 21:6330–6337. [PubMed: 12456640]
- Michel K, Budd A, Pinto S, Gibson TJ, Kafatos FC. *Anopheles gambiae* SRPN2 facilitates midgut invasion by the malaria parasite *Plasmodium berghei*. *EMBO Rep.* 2005; 6:891–897. [PubMed: 16113656]
- Michel K, Suwanchaichinda C, Morlais I, Lambrechts L, Cohuet A, Awono-Ambene PH, Simard F, Fontenille D, Kanost MR, Kafatos FC. Increased melanizing activity in *Anopheles gambiae* does not affect development of *Plasmodium falciparum*. *PNAS.* 2006; 103:16858–16863. [PubMed: 17065316]
- Miura K, Keister D, Muratova O, Sattabongkot J, Long C, Saul A. Transmission-blocking activity induced by malaria vaccine candidates Pfs25/Pvs25 is a direct and predictable function of antibody titer. *Malaria J.* 2007; 6:107.
- Murray CJL, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, Fullman N, Naghavi M, Lozano R, Lopez AD. Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet.* 2012; 379:413–431. [PubMed: 22305225]
- Rodrigues J, Oliveira GA, Kotsyfakis M, Dixit R, Molina-Cruz A, Jochim R, Barillas-Mury C. An Epithelial Serine Protease, AgESP, Is Required for *Plasmodium* Invasion in the Mosquito *Anopheles gambiae*. *PLoS ONE.* 2012; 7:e35210. [PubMed: 22509400]
- Rosenberg R. Malaria: some considerations regarding parasite productivity. *Trends Parasitol.* 2008; 24:487–491. [PubMed: 18805735]
- Sheehy SH, Duncan CJA, Elias SC, Choudhary P, Biswas S, Halstead FD, Collins KA, Edwards NJ, Douglas AD, Anagnostou NA, Ewer KJ, Havelock T, Mahungu T, Bliss CM, Miura K, Poulton ID, Lillie PJ, Antrobus RD, Berrie E, Moyle S, Gantlett K, Colloca S, Cortese R, Long CA, Sinden RE, Gilbert SC, Lawrie AM, Doherty T, Faust SN, Nicosia A, Hill AVS, Draper SJ. ChAd63-MVA-vectored Blood-stage Malaria Vaccines Targeting MSP1 and AMA1: Assessment of Efficacy Against Mosquito Bite Challenge in Humans. *Mol. Ther.* 2012; 20:2355–2368. [PubMed: 23089736]
- Sinden R. A biologist's perspective on malaria vaccine development. *Human Vaccines.* 2010; 6:3–11. [PubMed: 19946205]
- The malERA Consultative Group on Vaccines. *PLoS Med.* Public Library of Science; 2011. A Research Agenda for Malaria Eradication: Vaccines; p. e1000398

Highlights

- Vaccines that prevent transmission of *Plasmodium* parasites from mosquitoes would help towards eradicating malaria
- Most such transmission-blocking vaccines target parasite antigens expressed within the mosquito
- A vaccine is described which induced antibodies against a regulatory component of the mosquito innate immune response
- These antibodies reduce the intensity of *Plasmodium berghei* infection in mosquitoes
- This may be a novel approach to malaria transmission-blocking vaccines

Fig. 1. *Anopheles gambiae* serpin-2 (AgSRPN2)-specific total IgG responses following immunization. BALB/c mice ($n = 5$) were immunized with Ad-MVA AgSRPN2 (ChAd63 AgSRPN2 prime, MVA AgSRPN2 boost). Total IgG responses against recombinant AgSRPN2 protein were measured by ELISA in the serum of mice taken at the number of days following first immunization as shown. The arrow indicates the day of the boosting immunization.

Fig. 2.

Oocyst numbers in *Anopheles stephensi* mosquitoes 11 days after feeding on *Plasmodium berghei*-infected blood (direct membrane feeding assay, DMFA) in the presence of sera pooled from mice ($n = 8$) immunized with either *Anopheles gambiae* serpin-2 (AgSRPN2) or an irrelevant control antigen (GFP). Oocyst intensity data were best described by a zero-inflated negative binomial error structure. CI, confidence interval.

Fig. 3.

Results of direct feeding assays with *Plasmodium berghei*-infected mice and standard membrane feeding assays with *Plasmodium falciparum*. (A) Oocyst numbers in *Anopheles stephensi* mosquitoes 11 days after feeding on either mice immunized with *Anopheles gambiae* serpin-2 (AgSRPN2) (bars indicate mean, $n = 5$), or an irrelevant control antigen (bars indicate mean, $n = 4$). (B) Combined oocyst numbers from immunized mice presented in A. Oocyst intensity data were best described by a negative binomial error structure. (C) Mean oocyst numbers in *A. stephensi* mosquitoes 8 days after feeding on cultured *P. falciparum* gametocytes in a standard membrane feeding assay (SMFA). Oocyst intensity data were best described by a negative binomial error structure. CI, confidence interval.