Published in final edited form as:

Expert Rev Anti Infect Ther. 2015 August ; 13(8): 923–926. doi:10.1586/14787210.2015.1058709.

Circumsporozoite protein as a potential target for antimalarials

Cristina Fernández-Arias, Sara Mashoof, Jing Huang, and Moriya Tsuji

HIV and Malaria Vaccine Program, Aaron Diamond AIDS Research Center, Affiliate of The Rockefeller University, New York, NY, USA

Abstract

Since the discovery of circumsporozoite protein (CSP), a major sporozoite surface antigen, by Ruth and Victor Nussenzweigs in the early 1980s, the role of CSP in protection against malaria has been extensively investigated. Several monoclonal antibodies against CSP have been generated to date, with some of them mediating anti-malaria protection upon passive transfer into animals. Genetically engineered transgenic mosquitoes producing the anti-CSP antibody have recently been generated to reduce malaria transmission. A monoclonal anti-CSP antibody was produced in mice by adeno-associated virus vector and protected them from malaria. Phase III trials with RTS,S vaccine that targets CSP of *Plasmodium falciparum* have shown modest efficacy. Polyclonal anti-CSP antibodies derived from children who received the RTS,S vaccine failed to block malaria transmission through mosquitoes, but passive transfer of monoclonal antibodies raised from RTS,S-vaccinated recipient, conferred protection against malaria in mice. Taken together, these findings may imply CSP as an antimalarial target.

Keywords

malaria; plasmodium; circumsporozoite protein; sporozoites; monoclonal antibody; protection

Discovery of CSP

Circumsporozoite protein (CSP) was first discovered as a major surface protein of *Plasmodium berghei* sporozoites by a group led by Ruth S. Nussenzweig and Victor Nussenzweig [1–3]. Soon thereafter, CSPs of other plasmodial species were identified [4–10] and were shown to have similar structural and immunological properties. CSP with the size of approximately 40–60 kDa contains random repeats of an immunodominant B cell epitope [5–15] surrounded by N-terminal and C-terminal domains.

Generation of monoclonal antibodies against CSP

Several monoclonal antibodies have been raised against CSPs of various plasmodial species by different investigators, and most of them have been shown to recognize the

* Author for correspondence: Moriya Tsuji, Tel: 1-212-448-5021, mtsuiji@adarc.org.

Financial & competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript, apart from those disclosed.

immunodominant repeat domain of CSP [2–4, 16–26] and could neutralize parasite infectivity *in vitro* [17, 19, 23], and in some cases, *in vivo* [1–3, 18, 20, 21]. It is noteworthy that one study successfully isolated a monoclonal antibody against CSP from *P. falciparum* sporozoite-immunized individual by way of a phage display library [23]. A handful of monoclonal antibodies raised against CSP have been shown to recognize the non-repeat regions [24–30]. Some of these monoclonal antibodies were raised against either the C-terminus [24–27] or the N-terminus [24, 27, 28] region of CSP. Using a panel of monoclonal antibodies that recognize the C-terminus and repeat regions of *P. falciparum* CSP, the structure of CSP was revealed to be an elongated, flexible, rod-like protein [26]. A monoclonal antibody against the N-terminus of CSP appears to be involved in the processing of CSP, which was shown to neutralize sporozoite infectivity *in vivo* [28]. In one study, a series of monoclonal antibodies that recognize a processing-dependent epitope of CSP were generated. These antibodies recognize the epitope present in sporozoites of not only *P. berghei*, but also others, including *P. falciparum*; the antibodies have also inhibited *P. berghei* sporozoite invasion of hepatoma cells *in vitro*, but failed to neutralize its infectivity *in vivo* [29].

Production of the anti-CSP antibody in the mosquito, a vector for malaria

There have been several attempts to produce anti-CSP antibodies in mosquitoes, a vector for malaria. In an earlier study, Sindbis virus expressing a single-chain Fv (scFv) or a monoclonal anti-CSP antibody of *P. gallinaceum*, which is an avian malaria parasite, was constructed by James' group. *Aedes aegypti* mosquitoes were infected by the recombinant Sindbis virus to transduce the single-chain variable fragment (scFv) of the anti-CSP antibody into their salivary glands [31]. The expression of the scFv of a monoclonal anti-CSP antibody was able to almost completely reduce the sporozoite infection of salivary glands. More recently, two independent research groups have constructed transgenic mosquitoes that produce the scFv of 2A10, a monoclonal antibody against (NANP)_n of *P. falciparum* [32, 33]. A group led by Anthony James produced a scFv of a monoclonal antibody against a sexual stage antigen, either Chitinase 1 or Pfs25, of the parasite, in addition to the scFv of 2A10, in transgenic mosquitoes. The expression of a single copy of the dual scFv transgenes in mosquitoes was found to completely inhibit the development of *P. falciparum* parasites without imposing a fitness cost on the mosquitoes [32]. Sumitani et al. had also constructed transgenic mosquitoes expressing the scFv of 2A10 in their salivary glands; this group showed that the transmission of transgenic *P. berghei* sporozoites, rodent parasites that express *P. falciparum* CSP, from mosquitoes to mice was significantly reduced [33].

Production of the anti-CSP antibody in the mammalian host, the mouse

Ketner's group has most recently achieved the production of a monoclonal antibody against CSP in a mammalian host, using adeno-associated virus (AAV)-based gene transfer technology [34]. In this study, mice were first transduced with the gene encoding 2A10 monoclonal antibody against *P. falciparum* CSP by an AAV-mediated gene transfer. Next, by challenging the transduced and non-transduced mice with transgenic *P. berghei* parasites

expressing *P. falciparum* CSP, only the transduced mice were found to be protected against malaria.

Role of the anti-CSP antibody induced by the RTS,S vaccine

Until now, the most promising malaria vaccine candidate has been the RTS,S/AS01 (RTS,S) vaccine, which consists of a portion of the CSP of *P. falciparum* fused to a viral envelope protein of the hepatitis B virus [35–37]. Recent Phase III trials with the RTS,S vaccine candidate in children have demonstrated modest efficacy against clinical and severe malaria [35–37]. In an attempt to determine whether the anti-CSP antibodies induced by the RTS,S vaccine in humans can inhibit malaria transmission, Miura et al. purified polyclonal human anti-CSP antibodies from the pools of sera collected from RTS,S-vaccinated children and fed them to mosquitoes [38]. It was found that the anti-CSP antibodies failed to inhibit *P. falciparum* oocyst formation and/or sporogony in the mosquito host, which indicates a negligible role for RTS,S vaccine-induced anti-CSP antibodies in reducing malaria transmission. In another study done by Foquet et al., monoclonal anti-CSP antibodies were generated from an RTS,S-vaccinated recipient, and passively transferred to mice that were subsequently challenged with transgenic *P. berghei* parasites expressing *P. falciparum* CSP; these monoclonal anti-CSP antibodies were able to confer antimalarial protection [39]. Therefore, it appears that anti-CSP antibodies induced by the RTS,S vaccine might play a role in inhibiting the development of the pre-erythrocytic stages of malaria, but not its transmission.

Concluding remarks

The role of humoral immunity against malaria has been demonstrated as early as the 1960s by a study, in which passive transfer of human IgG from adults living in hyper-endemic areas to children was conducted and shown to protect the children from contracting malaria for a short period of time [40]. After the discovery of the major sporozoite antigen, CSP, the characterization and protective role of CSP have been extensively investigated [41]. In recent years, the development of a malaria vaccine, RTS,S, which is based on CSP, has reached a culmination point. Indeed, large-scale Phase III trials have just completed and shown a modest efficacy of a CSP-based malaria vaccine among children in Africa [35–37]. Antibodies against CSP are now considered a tool not only for protecting humans from malarial infection, but also for blocking malarial transmission. An attempt to produce a monoclonal anti-CSP antibody in a mammalian host, using a vector-mediated gene transfer approach, might turn out to be useful in the future for protecting a small number of travelers or military personnel deployed to malaria-endemic areas. However, it is highly costly and technically challenging to mass-produce the GMP-grade, humanized anti-CSP monoclonal antibody even for a small number of people. Thus, more in-depth studies are required before CSP can be considered a potential target for antimalarials in the clinic.

Acknowledgments

This work was funded by the National Institutes of Health, National Institute of Allergy, and Infectious Diseases Grants R01 AI070258 and R01 AI102891 (both to M.T.).

Biographies

Fernandez

Mashoof

Huang

Tsuji

References

1. Yoshida N, Nussenzweig RS, Potocnjak P, et al. Hybridoma produces protective antibodies directed against the sporozoite stage of malaria parasite. *Science*. 1980; 207(4426):71–73. [PubMed: 6985745]
2. Potocnjak P, Yoshida N, Nussenzweig RS, et al. Monovalent fragments (Fab) of monoclonal antibodies to a sporozoite surface antigen (Pb44) protect mice against malarial infection. *J Exp Med*. 1980; 151(6):1504–1513. [PubMed: 6991628]
3. Yoshida N, Potocnjak P, Nussenzweig V, et al. Biosynthesis of Pb44, the protective antigen of sporozoites of *Plasmodium berghei*. *J Exp Med*. 1981; 154(4):1225–1236. [PubMed: 7026723]
4. Nardin EH, Nussenzweig V, Nussenzweig RS, et al. Circumsporozoite proteins of human malaria parasites *Plasmodium falciparum* and *Plasmodium vivax*. *J Exp Med*. 1982; 156(1):20–30. [PubMed: 7045272]
5. Godson GN, Ellis J, Svec P, et al. Identification and chemical synthesis of a tandemly repeated immunogenic region of *Plasmodium knowlesi* circumsporozoite protein. *Nature*. 1983; 305(5929): 29–33. [PubMed: 6193427]

6. Ozaki LS, Svec P, Nussenzweig RS, et al. Structure of the plasmodium knowlesi gene coding for the circumsporozoite protein. *Cell*. 1983; 34(3):815–822. [PubMed: 6313209]
7. Enea V, Ellis J, Zavala F, et al. DNA cloning of Plasmodium falciparum circumsporozoite gene: amino acid sequence of repetitive epitope. *Science*. 1984; 225(4662):628–630. [PubMed: 6204384]
8. Enea V, Arnot D, Schmidt EC, et al. Circumsporozoite gene of plasmodium cynomolgi (Gombak):cDNA cloning and expression of the repetitive circumsporozoite epitope. *Proc Natl Acad Sci U S A*. 1984; 81(23):7520–7524. [PubMed: 6209717]
9. Arnot DE, Barnwell JW, Tam JP, et al. Circumsporozoite protein of Plasmodium vivax: gene cloning and characterization of the immunodominant epitope. *Science*. 1985; 230(4727):815–818. [PubMed: 2414847]
10. Dame JB, Williams JL, McCutchan TF, et al. Structure of the gene encoding the immunodominant surface antigen on the sporozoite of the human malaria parasite Plasmodium falciparum. *Science*. 1984; 225(4662):593–599. [PubMed: 6204383]
11. McCutchan TF, Lal AA, de la Cruz VF, et al. Sequence of the immunodominant epitope for the surface protein on sporozoites of Plasmodium vivax. *Science*. 1985; 230(4732):1381–1383. [PubMed: 2416057]
12. Eichinger DJ, Arnot DE, Tam JP, et al. Circumsporozoite protein of Plasmodium berghei: gene cloning and identification of the immunodominant epitopes. *Mol Cell Biol*. 1986; 6(11):3965–3972. [PubMed: 2432395]
13. Lal AA, de la Cruz VF, Welsh JA, et al. Structure of the gene encoding the circumsporozoite protein of Plasmodium yoelii. A rodent model for examining antimalarial sporozoite vaccines. *J Biol Chem*. 1987; 262(7):2937–2940. [PubMed: 3102479]
14. Procell PM, Collins WE, Campbell GH. Circumsporozoite protein of the human malaria parasite Plasmodium ovale identified with monoclonal antibodies. *Infect Immun*. 1988; 56(2):376–379. [PubMed: 3338845]
15. Krettli AU, Rocha EM, Lopes JD, et al. Circumsporozoite protein of Plasmodium gallinaceum characterized by monoclonal antibodies. *Parasite Immunol*. 1988; 10(5):523–533. [PubMed: 2461541]
16. Cochrane AH, Collins WE, Nussenzweig RS. Monoclonal antibody identifies circumsporozoite protein of Plasmodium malariae and detects a common epitope on Plasmodium brasilianum sporozoites. *Infect Immun*. 1984; 45(3):592–595. [PubMed: 6381308]
17. Hollingdale MR, Nardin EH, Tharavanij S, et al. Inhibition of entry of Plasmodium falciparum and P. vivax sporozoites into cultured cells in an in vitro assay of protective antibodies. *J Immunol*. 1984; 132(2):909–913. [PubMed: 6317752]
18. Charoenvit Y, Mellouk S, Cole C, et al. Monoclonal, but not polyclonal, antibodies protect against Plasmodium yoelii sporozoites. *J Immunol*. 1991; 146(3):1020–1025. [PubMed: 1988490]
19. Del Giudice G, Tougne C, Renia L, et al. Characterization of murine monoclonal antibodies against a repetitive synthetic peptide from the circumsporozoite protein of the human malaria parasite, Plasmodium falciparum. *Mol Immunol*. 1991; 28(9):1003–1009. [PubMed: 1922106]
20. Sina BJ, Wright C, Ballou R, et al. A protective monoclonal antibody with dual specificity for Plasmodium falciparum and Plasmodium berghei circumsporozoite proteins. *Exp Parasitol*. 1992; 74(4):431–440. [PubMed: 1375561]
21. Ak M, Bower JH, Hoffman SL, et al. Monoclonal antibodies of three different immunoglobulin G isotypes produced by immunization with a synthetic peptide or native protein protect mice against challenge with Plasmodium yoelii sporozoites. *Infect Immun*. 1993; 61(6):2493–2497. [PubMed: 8500885]
22. Udhayakumar V, Qari SH, Patterson P, et al. Monoclonal antibodies to the circumsporozoite protein repeats of a Plasmodium vivax-like human malaria parasite and Plasmodium simiovale. *Infect Immun*. 1994; 62(5):2098–2100. [PubMed: 8168975]
23. Chappel JA, Hollingdale MR, Kang AS. IgG(4) Pf NPNA-1 a human anti-Plasmodium falciparum sporozoite monoclonal antibody cloned from a protected individual inhibits parasite invasion of hepatocytes. *Hum Antibodies*. 2004; 13(3):91–96. [PubMed: 15598989]
24. Stüber D, Bannwarth W, Pink JR, et al. New B cell epitopes in the Plasmodium falciparum malaria circumsporozoite protein. *Eur J Immunol*. 1990; 20(4):819–824. [PubMed: 1693336]

25. Burkot TR, Da ZW, Geysen HM, et al. Fine specificities of monoclonal antibodies against the *Plasmodium falciparum* circumsporozoite protein: recognition of both repetitive and non-repetitive regions. *Parasite Immunol.* 1991; 13(2):161–170. [PubMed: 2052404]
26. Plassmeyer ML, Reiter K, Shimp RL Jr, et al. Structure of the *Plasmodium falciparum* circumsporozoite protein, a leading malaria vaccine candidate. *J Biol Chem.* 2009; 284(39): 26951–26963. [PubMed: 19633296]
27. Rocha EM, Hollingdale MR, Sina B, et al. Common epitopes in the circumsporozoite proteins of *Plasmodium berghei* and *Plasmodium gallinaceum* identified by monoclonal antibodies to the *P. gallinaceum* circumsporozoite protein. *J Eukaryot Microbiol.* 1993; 40(1):61–63. [PubMed: 7681341]
28. Espinosa DA, Gutierrez GM, Rojas-López M, et al. Proteolytic Cleavage of the *Plasmodium falciparum* Circumsporozoite Protein Is a Target of Protective Antibodies. *J Infect Dis.* 2015 pii: jiv154. [Epub ahead of print].
29. Tsuji M, Corradin G, Zavala F. Monoclonal antibodies recognize a processing dependent epitope present in the mature CS protein of various plasmodial species. *Parasite Immunol.* 1992; 14(5): 457–469. [PubMed: 1279504]
30. Gonzalez-Ceron L, Rodriguez MH, Wirtz RA, et al. *Plasmodium vivax*: a monoclonal antibody recognizes a circumsporozoite protein precursor on the sporozoite surface. *Exp Parasitol.* 1998; 90(3):203–211. [PubMed: 9806864]
31. de Lara Capurro M, Coleman J, Beerntsen BT, et al. Virus-expressed, recombinant single-chain antibody blocks sporozoite infection of salivary glands in *Plasmodium gallinaceum*-infected *Aedes aegypti*. *Am J Trop Med Hyg.* 2000; 62(4):427–433. [PubMed: 11220756]
32. Isaacs AT, Jasinskiene N, Tretiakov M, et al. Transgenic *Anopheles stephensi* coexpressing single-chain antibodies resist *Plasmodium falciparum* development. *Proc Natl Acad Sci U S A.* 2012; 109(28):E1922–E1930. [PubMed: 22689959]
33. Sumitani M, Kasashima K, Yamamoto DS, et al. Reduction of malaria transmission by transgenic mosquitoes expressing an ant sporozoite antibody in their salivary glands. *Insect Mol Biol.* 2013; 22(1):41–51. [PubMed: 23176559]
34. Deal C, Balazs AB, Espinosa DA, et al. Vectored antibody gene delivery protects against *Plasmodium falciparum* sporozoite challenge in mice. *Proc Natl Acad Sci U S A.* 2014; 111(34): 12528–12532. [PubMed: 25114213]
35. Agnandji ST, Lell B, et al. RTS,S Clinical Trials Partnership. A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. *N Engl J Med.* 2012; 367(24):2284–2295. [PubMed: 23136909]
36. Campo JJ, Sacarlal J, Aponte JJ, et al. Duration of vaccine efficacy against malaria: 5th year of follow-up in children vaccinated with RTS,S/AS02 in Mozambique. *Vaccine.* 2014; 32(19):2209–2216. [PubMed: 24631081]
37. RTS,S Clinical Trials Partnership. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet.* 2015 pii: S0140-6736(15)60721-8.
38. Miura K, Jongert E, Deng B, et al. Effect of ingested human antibodies induced by RTS S/AS01 malaria vaccination in children on *Plasmodium falciparum* oocyst formation and sporogony in mosquitoes. *Malar J.* 2014; 13:263. [PubMed: 25007730]
39. Foquet L, Hermesen CC, van Gemert GJ, et al. Vaccine-induced monoclonal antibodies targeting circumsporozoite protein prevent *Plasmodium falciparum* infection. *J Clin Invest.* 2014; 124(1): 140–144. [PubMed: 24292709]
40. Cohen S, McGregor IA, Carrington S. Gamma-globulin and acquired immunity to human malaria. *Nature.* 1961; 192:733–737. [PubMed: 13880318]
41. Nussenzweig V, Nussenzweig RS. Circumsporozoite proteins of malaria parasites. *Cell.* 1985; 42(2):401–403. [PubMed: 2411417]

Table 1
Epitope-specificity, Ig isotypes and neutralizing activity of monoclonal antibodies against *P. falciparum* CSP

Antigen	Epitope	Name of Mabs	Neutralizing Activity	Isotype	Origin	Reference
PfCS	repeat	1E9, 2A10, 2C11, 2E7, 3D6	<i>in vitro</i> *	IgG1	Mouse	4, 17
		CT1, CT2.1, CT2.2, CT2.3, CT3.1	<i>in vitro</i> *	IgM except CT3.1 (IgG1)		19
		1G2	<i>in vitro</i> *	IgG1		26
		1G3.4, 5C1, 5G5.3	Not Determined (ND)	IgG1		25
		PfNPN-1	<i>in vitro</i> *	IgG4		23
	non-repeat	Mal1C, Mal2A, Mal2B	<i>in vivo</i> **	ND	Human	39
		2A6, 2C7	ND	IgG1		24
		1B2.2, 2F1.1	ND	1B2.2 (IgG1) 2F1.1 (IgM)		25
		Mab 36	<i>in vitro</i> # and <i>in vivo</i> ##	IgM		20
		V12, V16, V17	ND	IgG1 except V16 (G3)		24
	Processing-dependent	5G5.3	ND	IgG1	Mouse	25
		1G12, 4B3, 4C2	<i>in vitro</i> *	IgG1		26
		2A6, 2C7	ND	IgG1		24
		5D5	<i>in vivo</i> †	IgG1		28
		2B4, 5D9, 7G5	<i>in vitro</i> ††	IgM		29

* Infection of *P. falciparum* sporozoites to human hepatoma cells or human primary hepatocytes.

** Infection of *P. falciparum* sporozoites to uPA-SCID mice possessing human liver.

Mab36 reacts with CSP of both *P. falciparum* and *P. berghei*. Hence, infection of *P. falciparum* sporozoites or *P. berghei* sporozoites to human hepatoma cells, HepG2-A16.

Mab36 reacts with CSP of both *P. falciparum* and *P. berghei*. Hence, infection of *P. berghei* sporozoites to wild-type mice.

† Infection of transgenic *P. berghei* sporozoites expressing the N-terminus of *P. falciparum* CSP to wild-type mice.

†† 2B4, 5D9 and 7G5 react with CSP of both *P. falciparum* and *P. berghei*. Hence, infection of *P. berghei* sporozoites to human hepatoma cells, HepG2-A16.