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研究課題名（和文）Comprehensive analysis of antibody responses to Plasmodium falciparum repetitive interspersed family (RIFIN) proteins

研究課題名（英文）Comprehensive analysis of antibody responses to Plasmodium falciparum repetitive interspersed family (RIFIN) proteins

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交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：本研究はマラリア流行地住民の熱帯熱マラリアに対する免疫の標的タンパク質を同定するため実施した。まず66人のウガンダ人血清（6-22歳）と265種のマラリアタンパク質の反応性を調べ、98%以上のタンパク質に対する抗体を保有することが分かった。さらに特定のタンパク質に対する抗体量が多い小児ではマラリアの発症リスクが低かった。また、PF3D7_0801000タンパク質はウガンダ人に対する免疫原性が高く、年齢とともに抗体の量が増加し、さらにその抗体はin vitro実験でマラリア原虫の赤血球侵入を阻害することが分かった。本研究で同定したタンパク質は、発症阻止ワクチン候補として今後研究を進めていく。

研究成果の学術的意義や社会的意義
The need for a vaccine against malaria is urgent. Emergence of SARS-CoV-2 COVID-19, has greatly interrupted malaria control efforts. The findings in this study directly support malaria vaccine studies towards protecting residents of the malaria endemic countries as well as travelers to this regions.

研究成果の概要（英文）：The aim of this study was to identify the proteins that are targets of naturally acquired immunity against Plasmodium falciparum malaria in individuals residing in a malaria endemic regions. In the initial phase, we observed that >98% of the 265 proteins that were assayed were immunogenic in malaria-exposed individuals in Uganda. Meaning, children had antibodies to these proteins. Additionally, children with high levels of antibodies to some proteins (4 RIFINs, a STEVOR, and a SURFIN 1.2) had lower risk of developing clinical malaria. Subsequently, we observed that antibodies to a potential vaccine antigen, PF3D7_0801000 which localizes in parasite merozoites, blocks malaria parasite growth in in vitro cultures. The protein is strongly immunoreactive with serum of malaria exposed individuals, and antibodies are acquired with increasing with age. These selected proteins need further evaluation as asexual blood-stage vaccine candidate antigens.

研究分野：Malaria, マラリア

キーワード：マラリア　Vaccine　RIFIN　SURFIN　STEVOR　PF3D7_0801000

１．研究開始当初の背景

P. falciparum malaria afflicts an about 200 million people annually with an estimated 430,000 deaths in 2016; of which 90% are in sub-Saharan Africa. Development of drug resistance, to both parasites and mosquito vectors, underscores the need to improve existing tools and develop new approaches including vaccines geared toward malaria elimination. Association between levels of antibodies and the risk of clinical malaria have pointed to an important role of *P. falciparum* antigens.

Antibodies against variable surface antigens (VSA) that are expressed on the surface of infected erythrocytes could be useful targets for anti-malaria therapeutics and/or vaccines. Among the VSAs, several PfEMP1, RIFINs, STEVORs and SURFINs have been demonstrated as the target of naturally acquired immunity against malaria. This is in people who are residents of malaria endemic regions. For instance, several studies examining the association between levels of antibodies targeting some RIFINs and the risk of clinical malaria have pointed to an important role these *P. falciparum* antigens in blocking development of symptomatic malaria; which includes fever and high parasitemia. Indeed, RIFIN family members are important ligands for opsonization of *P. falciparum* infected erythrocyte with specific immunoglobulins (IgG) acquiring broad protective reactivity. However, the global repertoire of human anti-VSAs IgG, its variation in children, and the key protective targets remain poorly understood. This has mainly been due to challenges associated with expression of VSAs making comprehensive immune responses evaluation a daunting task. Application of wheat germ cell-free system could help overcomes these limitations.

２．研究の目的

The major objective of this study was to determine the RIFINs and other proteins whose antibody levels are predictive of protection from symptomatic malaria (clinical immunity) in Ugandan children and young adults, that are continually exposed to malaria. This is based on the strong evidence that protective immunity develops in individuals exposed to *P. falciparum* infections. Identification of key targets of human protective immunity would facilitate prioritization of antigens for further evaluation as biomarkers of immunity in malaria surveillance and control.

３．研究の方法

To answer the above question, we performed the steps below. Protein Library: We developed of a protein library consisting of RIFINs (n=178), STEVORs (n=54), and SURFINs (n=33). The recombinant proteins (n=265) derived from *P. falciparum* 3D7, were expressed as mono-biotinylated molecules using the wheat germ cell-free system; a robust eukaryotic protein expression system. Expression was confirmed using Western blot by probing with HRP conjugated

streptavidin. Quantitation of antigen-specific antibodies by AlphaScreen: Using the developed protein library, we measured antibodies in serum samples collected in a prospective study of 66 participants aged 6-20 years who were residing in Northern Uganda. All AlphaScreen assays were conducted in a randomized manner to avoid batch bias. Data analysis: Subsequently, to assess associations between antibody levels and the prospective risk of developing symptomatic malaria, the generated data was analyzed using an R software (R Foundation for Statistical Computing) based large data analysis platform.

４．研究成果

We report that wheat germ cell-free system could successfully express all the RIFINs, STEVORs and SURFINs derived from *P. falciparum* 3D7 parasite strain. This allowed comprehensive serological profiling of serum obtained from a malaria exposed population in Uganda. We observed that >98% assayed proteins (n=265) were immunogenic in the malaria naturally exposed individuals. The overall breadth (number of reactive antigens per person) of immune response associated with individuals age but not symptomatic malaria outcome. However, children with high levels of antibodies to some proteins (four RIFINs (PF3D7_0201000, PF3D7_1254500, PF3D7_1040600, PF3D7_1041100), STEVOR (PF3D7_0732000), and SURFIN 1.2 (PF3D7_0113600) reduced risk of developing symptomatic malaria during the study follow-up period, suggesting the 5 antigens are important targets of protective immunity against clinical malaria. In addition, we observed that antibodies to a PF3D7_0801000, potential vaccine antigen, which localizes in parasite merozoites, blocks malaria parasite growth in *in vitro* cultures. The protein is strongly immunoreactive with serum of malaria exposed individuals, and antibodies are acquired with increasing with age. Taken together, these findings have implications for understanding malaria susceptibility, immunity and vaccine development studies. The selected proteins need further evaluation for their potency as asexual blood-stage vaccine antigens, a consideration we are pursuing now.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　3件）

１．著者名 Kanoi Bernard N.、Nagaoka Hikaru、White Michael T.、Morita Masayuki、Palacpac Nirianne M. Q.、Ntege Edward H.、Balikagala Betty、Yeka Adoke、Egwang Thomas G.、Horii Toshihiro、Tsuboi Takafumi、Takashima Eizo	４．巻 11
２．論文標題 Global Repertoire of Human Antibodies Against Plasmodium falciparum RIFINs, SURFINs, and STEVORs in a Malaria Exposed Population	５．発行年 2020年
３．雑誌名 Frontiers in Immunology	６．最初と最後の頁 "
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fimmu.2020.00893	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Nagaoka Hikaru、Kanoi Bernard N.、Jinoka Kana、Morita Masayuki、Arumugam Thangavelu U.、Palacpac Nirianne M. Q.、Egwang Thomas G.、Horii Toshihiro、Tsuboi Takafumi、Takashima Eizo	４．巻 10
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３．雑誌名 Frontiers in Immunology	６．最初と最後の頁 "
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fimmu.2019.02669	査読の有無 有
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１．著者名 Kanoi Bernard N.、Nagaoka Hikaru、Morita Masayuki、Tsuboi Takafumi、Takashima Eizo	４．巻 80
２．論文標題 Leveraging the wheat germ cell-free protein synthesis system to accelerate malaria vaccine development	５．発行年 2021年
３．雑誌名 Parasitology International	６．最初と最後の頁 102224～102224
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.parint.2020.102224	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Nagaoka Hikaru、Kanoi Bernard N.、Morita Masayuki、Nakata Takahiro、Palacpac Nirianne M.Q.、Egwang Thomas G.、Horii Toshihiro、Tsuboi Takafumi、Takashima Eizo	４．巻 80
２．論文標題 Characterization of a Plasmodium falciparum PHISTc protein, PF3D7_0801000, in blood- stage malaria parasites	５．発行年 2021年
３．雑誌名 Parasitology International	６．最初と最後の頁 102240～102240
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.parint.2020.102240	査読の有無 有
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2．発表標題 Human antibody repertoires to RIFINs and STEVORs associate with reduced risk to febrile malaria
3．学会等名 American Society of Tropical Medicine and Hygiene 68th Annual Meeting, 20th-24th, November 2019, National Harbor, Maryland, USA.（国際学会）
4．発表年 2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

7．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

8．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関