様式Ｃ－１９

科学研究費助成事業（科学研究費補助金）研究成果報告書

平成　24 年　5 月　25 日現在

機関番号：14401
研究種目：若手研究（Ｂ）
研究期間：2010～2011 年度
課題番号：22790394
研究課題名（和文）マラリア感染免疫におけるリポカリンの役割

研究課題名（英文）Role of Lipocalin 2 during malaria infection

研究代表者
Coban Cevayir（チョバン　ジェヴァイア）
大阪大学・免疫学フロンティア研究センター・特任准教授
研究者番号：００３９７７１２

研究成果の概要（和文）：

研究成果の概要（英文）：
Malaria is one of the most deadly infectious diseases in the world, with no effective vaccine yet that many of the host immune responses against malaria parasites remain unclear. *Plasmodium* parasites feed and multiply within erythrocytes, and their egress from these cells results in various degrees of anemia. Little is known about how host iron homeostasis affects the immune system during malaria. In this research we found that Lipocalin 2, a known early innate immune response molecule, is abundantly secreted during mouse malaria infection, and is required for the clearance of parasites. Therefore, results of this research may lead to a new understanding of Lcn2-mediated anti-malarial immunity through the modulation of host iron metabolism.

交付決定額

（金額単位：円）

	直接経費	間接経費	合　計
2010 年度	2,000,000	600,000	2,600,000
2011 年度	700,000	210,000	910,000
年度			
総　計	2,700,000	810,000	3,510,000

研究分野：医歯薬学
科研費の分科・細目：基礎医学・寄生虫学
キーワード：マラリア，リポカン２，鉄代謝，感染症,貧血

１．研究開始当初の背景

Our laboratory has been investigating the role of innate immune system during malaria infection that we could point-out several molecules important for host defense against malaria parasites (*Coban, C et al Int. Immunol. 2007; Coban, C et al Trends in Microbiology. 2007; Coban C, et al J Exp Med. 2005*). We have reported that MyD88/TLR9/TLR2 pathway has important role on the pathogenesis of cerebral malaria in mice. Similarly, we have shown that malarial metabolite hemozoin is a TLR9 ligand that we’ve pursued extensive studies to use hemozin as a new vaccine adjuvant (Coban et al., Cell Host Microbe, 2010). In our previous report we’ve found in a micro-array analysis that Lipocalin 2 (Lcn2) (also called neutrophil gelatinase-associated lipocalin or NGAL

or 24p3) gene was strongly up-regulated in the brains of mice with cerebral malaria (*Coban, C et al Int. Immunol. 2007*). Lcn2 has been known to mediate host defense against bacteria through sequestration of iron. Iron is very important for the growth of most of the microorganisms including malaria parasites. However, little is known how host iron homeostasis affects the immune system during malaria.

２．研究の目的

In this project, we aimed to characterize the role of antimicrobial protein Lcn2 against malaria infection by using animal models of malaria infection in mice. We hypothesized that Lcn2 may regulate the interactions between iron homeostasis and the immune system during (blood-stage) malaria infection.

３．研究の方法

a) *Lcn2*$^{-/-}$ mice were generated on a 129/Ola X C57Bl/6 (B6.129) background and backcrossed to B6 and Balb/c mice (CLEA, Japan) for at least 9 generations. Age and gender matched wild type (WT) controls were purchased from CLEA, Japan.
b) Various *Plasmodium* infections in mice were performed. Parasitemia and reticulocytemia were assessed by microscopic counts of Giemsa-stained thin blood smears every two days. Blood hemoglobin levels were analyzed using Drabkin's reagent. Total blood cells were counted by a particle counter from the fresh heparinized blood.
c) Serum Lcn2 levels were measured by ELISA. Splenocytes and BM cells were prepared and stained for flow cytometry. Total RNA was prepared from tissues for Lcn2,
d) Immunohistochemistry was performed from the tissues. Tissue iron staining was performed by using iron staining kit.

４．研究成果

Several novel findings in this research includes;

a) We found that Lcn2, a known anti-bacterial siderocalin and a component of the innate immune system, is abundantly secreted into serum during blood-stage *Plasmodium* infection.
b) Lcn2 plays a critical role in controlling parasitemia.
c) Innate immune cells are the source of Lcn2.
d) Tissue iron levels were significantly affected in *Lcn2*$^{-/-}$ mice indicating that iron re-cycling was affected by Lcn2 deficiency during *Plasmodium* infection.

In summary, we have found that Lcn2 functions not only as an anti-microbial, but also as anti-plasmodial defense molecule.

５．主な発表論文等

（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計 13 件）

① Culleton R, Coban C, Yildiz Zeyrek F, Cravo P, Kaneko A, Randrianarivelojosia M, Andrianaranjaka V, Kano S, Farnert A, Arez A, Sharp PM , Carter R, Tanabe K. The origins of African *Plasmodium vivax*; insights from mitochondrial genome sequencing. PLoS ONE, 2011, 6(12): e29137. PEER REVIEWED.

② Yildiz Zeyrek F, Palacpac N, Yuksel F, Yagi M, Honjo K, Fujita Y, Arisue N, Takeo S, Tanabe K, Horii T, Tsuboi T, Ishii KJ, Coban C*. Serologic Markers in Relation to Parasite Exposure History Help to Estimate Transmission Dynamics of *Plasmodium vivax*. PLoS ONE, 2011, 6(11): e28126. (*corresponding author) PEER REVIEWED.

③ Coban C, Kobiyama K, Aoshi T, Takeshita F, Horii T, Akira S, Ishii KJ. Novel Strategies to Improve DNA Vaccine Immunogenicity. Review, Current Gene Therapy, 2011, Dec;11(6):479-484. PEER REVIEWED.

④ Marichal T, Ohata K, Bedoret D, Mesnil C, Sabatel C, Kobiyama K, Lekeux P, Coban C, Akira S, Ishii KJ, Bureau F, Desmet CJ. DNA released from dying host cells mediates aluminum adjuvant activity. Nature Medicine, 2011, Jul 17; 17(8):996-1002. PEER REVIEWED.

⑤ Kuroda E, Ishii KJ, Uematsu S, Ohata K, Coban C, Akira S, Aritake K, Urade Y, Morimoto Y. Silica Crystals and

Aluminum Salts Regulate the Production of Prostaglandin in Macrophages via NALP3 Inflammasome-Independent Mechanisms. Immunity, 2011, 34(4):514-526. PEER REVIEWED.

⑥ 津久井利広、Coban, Cevayir, 八木正典、大畑敬一、猪狩義勝、福井真人、堀井俊宏、審良静男、石井 健 「マラリアヘモゾインに対するＴＬＲ９での反応と免疫増強効果」臨床免疫・アレルギー科 (2011) 55(1) : 28-34. PEER REVIEWED.

⑦ Coban C, Ohata K, Igari Y, Kato M, Tsukui T. Novel Adjuvant: A nanocrystal from malaria parasites. Adjuvant (Edited by Kouichi Yamanishi and Ken J. Ishii), CMC Publishing, 2011, 2.7, p107-115. ISBN Code: 978-4-7813-0405-2. PEER REVIEWED.

⑧ Yildiz Zeyrek F, Tachibana S, Yuksel F, Doni N, Palacpac N, Arisue N, Horii T, Coban C, Tanabe K. Limited polymorphism of the Plasmodium vivax merozoite surface protein 1 gene in isolates from Turkey. American Journal of Tropical Medicine and Hygiene, 2010, 83(6):1230-1237. PEER REVIEWED.

⑨ Shokouhi B, Coban C, Hasirci V, Aydin E, Dhanasingh A, Shi N, Koyama S, Akira S, Zenke M, Sechi AS. Biomedical polymers subvert dendritic cell function and adhesion through the activation of multiple Toll-like receptor signaling cascades. Biomaterials, 2010, 31(22):5759-5771. PEER REVIEWED.

⑩ Coban C*, Horii T, Akira S, Ishii KJ. TLR9 and Endogenous Adjuvants of the Whole Blood-stage Malaria Vaccine. Review, Expert Reviews of Vaccines, 2010, in press. (*corresponding author) PEER REVIEWED.

⑪ Coban C*, Yagi M, Ohata K, Igari Y, Tsukui T, Horii T, Ishii KJ, Akira S. The malarial metabolite hemozoin and its potential use as a vaccine adjuvant. Review, Allergology International, 2010, 59 (2) in press (*corresponding author) PEER REVIEWED.

⑫ Coban C, Igari Y, Yagi M, Reimer T, Koyama S, Aoshi T, Ohata K, Tsukui T, Takeshita F, Sakurai K, Ikegami T, Nakagawa A, Horii T, Nuñez G, Ishii KJ, Akira S. Immunogenicity of Whole Parasite Vaccines Against Plasmodium falciparum Involves Malarial Hemozoin and Host TLR9. Cell Host and Microbe, 2010, 7(1):50-61. PEER REVIEWED.

〔学会発表〕(計 11 件)

① Coban C. Novel mechanism of nanocrystal adjuvants; lessons from malaria parasites and their metabolites. 第 81 回日本寄生虫学会大会, 2012 年 3 月 23－24, 兵庫医科大学（兵庫県.

② Coban C. Novel mechanism of nanocrystal adjuvants; lessons from malaria parasites and their metabolites. World Immune Regulation Meeting (WIRM)-VI, March 18-21, 2012, Davos, SWITZERLAND.

③ Coban C. Novel mechanism of nanocrystal adjuvants; lessons from malaria parasites and their metabolites. The 5th Immunoparasitology Meeting, 2012 年 3 月 1-2 日, 大阪大学（大阪府）.

④ Coban C, Zhao H, Yagi M, Aoshi T, Horii T, Ishii KJ, Akira S. Lipocalin 2 and the iron metabolism during malaria infection. (Oral Presentation). 第40回日本免疫学会学術集会, 2011年11月27-29日幕張メッセ（千葉県）.

⑤ Coban C. Lipocalin 2 and the iron metabolism during malaria infection. Nagasaki Symposium on Malaria Biology 2011 (II), 2011 年 11 月 16-17 日, 長崎大学（長崎県）.

⑥ Coban C. Innate nanocrsytals and their adjuvant properties. November 10th, 2011, Department Center for Infectious Medicine (CIM), Huddinge Campus, Karolinska Institute, Stockholm, SWEDEN.

⑦ Coban C. Innate Immune responses to malaria parasites. November 8th, 2011, Department of Microbiology, Tumor and Cell Biology (MTC), Solna Campus, Karolinska Institute, Stockholm, SWEDEN.

⑧ Coban C. Underlying mechanism of nanocrystals' s vaccine adjuvant properties. Advances in Immunology and Cancer Biology, April 15-17, 2011, Bogazici University, Istanbul, TURKEY.

⑨ Coban C. A Nanocrystal from Malaria Parasite Hemozoin: From Malaria Infection to Vaccine Adjuvant. International Vaccine Institute (IVI), October 20th, 2010, Seoul, KOREA.

〔その他〕
ホームページ等
http://www.ifrec.osaka-u.ac.jp/jpn/laboratory/malariaimmunology/index.php

６．研究組織
(1)研究代表者
COBAN Cevayir (チョバン　ジェヴァイア)
大阪大学・免疫学フロンティア研究センター・特任准教授
研究者番号：００３９７７１２

(2)研究分担者
(　　)

研究者番号：

(3)連携研究者
審良　静男 (AKIRA SHIZUO)
大阪大学・免疫学フロンティア研究センター・教授
研究者番号：５０１９２９１９

堀井　俊宏 (HORII TOSHIHIRO)
大阪大学・微生物病研究所・教授
研究者番号：８０１４２３０５

石井　健 (ISHII KEN)
大阪大学・免疫学フロンティア研究センター・教授
研究者番号：００４４８０８６